

Ref.: 2014-08108-01  
CWCh/PNi

An application  
to amend the *Australia New Zealand Food Standards Code* with a

**Serine protease from *Nocardiosis prasina*  
produced by a genetically modified *Bacillus  
licheniformis***

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July 16<sup>th</sup> 2014

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## EXECUTIVE SUMMARY

The present application seeks to amend Standard 1.3.3. - Processing Aids of the Australia New Zealand Food Standards Code (the Code) to approve a serine protease enzyme preparation produced by Novozymes A/S.

### ***Proposed change to Standard 1.3.3 - Processing Aids***

The table to clause 17, Permitted enzymes of Microbial Origin, is proposed to be amended to include a serine protease from *Nocardiopsis prasina* produced in a genetically modified strain of *Bacillus licheniformis*.

The application is applied for assessment by the general procedure.

### ***Description of enzyme preparation***

The enzyme is a serine protease with chymotrypsin specificity (EC 3.4.21.1), which hydrolyses peptide bonds in proteins resulting in smaller proteins and peptides of variable lengths. The enzyme is produced by submerged fermentation of a *Bacillus licheniformis* microorganism expressing a serine protease from *Nocardiopsis prasina*.

The commercial enzyme product, CTL3 conc BG, is a granulated enzyme preparation and complies with the JECFA recommended purity specifications for food-grade enzymes.

The producing micro-organism, *Bacillus licheniformis*, is absent from the commercial enzyme product.

### ***Use of the enzyme***

The serine protease is used as processing aid for partial or extensive hydrolysis of animal and vegetable proteins (such as casein, whey, gluten, and proteins from soy, corn, rice, peas, lentils, meat and fish) to be further used as ingredients in a variety of beverage and food products.

The enzyme is added during the food production process, where it performs its function. In the final food product the enzyme protein is denatured by high temperature, which means that the enzyme does not have any action or any function in the final food.

### ***Benefits***

Since the 1970s proteases have been increasingly used in various industrial food applications for hydrolysis of proteins. Protein hydrolysates can also be produced by acid and alkaline hydrolysis as well as by heat treatment.

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As compared with these alternatives, the benefits of the action of serine protease are:

- Higher yield of soluble proteins and peptides
- Mild process conditions
- Reduced amounts of salts compared to acid hydrolysed protein
- Protein hydrolysate with controlled peptide profile due to specificity of the enzyme
- Increased digestibility of proteins

### **Safety evaluation**

The safety of the strain has been thoroughly assessed:

- The enzyme preparation complies with international specifications ensuring absence of contamination by toxic substances or noxious microorganisms.
- The production organism has a long history of safe use as production strain for food grade enzyme preparations and is not known to produce any harmful metabolites.
- The genetic modifications in the production strain are well-characterized and safe and the integrated DNA (enzyme gene) has been shown to be stably maintained.
- Sequence homology assessment to known allergens and toxins shows that oral intake of the serine protease does not pose any food allergenic or toxic concern.
- Two mutagenicity studies show that the food enzyme is unable to damage the genetic material of living organisms.
- An oral toxicological study in rodents (a 13-weeks study), where groups of animals were given the food enzyme at very high doses, show, that all dose levels were generally well tolerated.

Furthermore, the safety of the serine protease preparation has been confirmed or is under consideration by external expert groups, as follows:

- Denmark: The enzyme preparation has been safety assessed according to the Guidelines for the evaluation of food enzymes (the Scientific Committee for Food, Commission of the European Communities, 1992). This resulted in the authorisation of the enzyme product by the Danish authorities.
- France: The enzyme has been positively evaluated by the French Authorities and has been included in The French order of October 19, 2006 on use of processing aids in the manufacture of certain foodstuff, as amended
- JECFA: The enzyme preparation has been positively evaluated in the 76th meeting of JECFA and has been allocated an Acceptable Daily Intake (ADI) "not specified".
- Mexico: The enzyme has been positively evaluated by COFEPRIS, however the amendment to the positive list is awaiting the next official update.
- Brazil: The enzyme has been positively evaluated by ANVISA, however the amendment to the positive list is awaiting the next official update, expected in 2014.

### **Conclusion**

Based on the Novozymes safety evaluation (confirmed by the above-mentioned bodies), we respectfully request the inclusion of this enzyme in the Table to clause 17 of Standard 1.3.3.; Permitted enzymes of Microbial origin.

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# INTRODUCTION

The present dossier describes a serine protease enzyme preparation produced by submerged fermentation of a *Bacillus licheniformis* microorganism, expressing a serine protease gene from *Nocardioopsis prasina*. The Novozymes A/S trade name used for the serine protease enzyme preparation is CTL3 conc BG.

The serine protease is used as processing aid for partial or extensive hydrolysis of animal and vegetable proteins (such as casein, whey, gluten, and proteins from soy, corn, rice, peas, lentils, meat and fish) to be further used as ingredients in a variety of beverage and food products.

The enzyme is a serine protease with chymotrypsin specificity (EC 3.4.21.1), which hydrolyses peptide bonds in proteins with preferential cleavage at tyrosine, phenylalanine, leucine and methionine resulting in smaller proteins and peptides of variable lengths.

The following sections describe in detail the construction of the genetically modified *Bacillus licheniformis* used as the production organism, the production process, the product specification, the application of the enzyme preparation and finally the safety evaluation of the product including the toxicology program, which has been carried out confirming the safety of the product for its intended use.

The documentation has been elaborated according to the Application Handbook from Food Standards Australia New Zealand as of September 1<sup>st</sup> 2013, applied as relevant for an enzyme application, i.e. outlining the following section:

- SECTION 3.1 – GENERAL REQUIREMENTS
- SECTION 3.3.2 – PROCESSING AIDS, subsections A, C, D, E, F.

**NB!** When reading this document it should be noticed that in some reports, the serine protease enzyme preparation is described by its internal production batch code PPA 26797.

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## SECTION 3.1, GENERAL REQUIREMENTS

### 3.1.1 Executive Summary

An Executive Summary is provided as a separate copy together with this application.

### 3.1.2 Applicant details

- (a) **Applicant's name/s**  
[REDACTED]
- (b) **Company/organisation name**  
Novozymes Australia Pty Ltd
- (c) **Address (street and postal)**  
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2151 NORTH ROCKS NSW, Australia
- (d) [REDACTED]
- (e) [REDACTED]
- (f) **Nature of applicant's business**  
Biotechnology
- (g) **Details of other individuals, companies or organisations associated with the application.**  
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### 3.1.3 Purpose of the application

This application is submitted to provide for amendment of the Australia New Zealand Food Standards Code - Standard 1.3.3 - Processing Aids, Table to clause 17 to include a serine protease from *Nocardiosis prasina* produced in a genetically modified strain of *Bacillus licheniformis*.

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## 3.1.4 Justification for the application

### ***The need for the proposed change***

The Table to clause 17 of Standard 1.3.3 contains a list of permitted enzymes of microbial origin. There are a number of approved proteases, including serine endopeptidases (EC 3.4.21.14 and EC 3.4.21.26) from different sources.

The Table to clause 15 of Standard 1.3.3 contains a list of permitted enzymes of animal origin. Also in this table there are a number of approved proteases from different animal sources.

However, none of the Tables contain a serine protease with chymotrypsin specificity (EC 3.4.21.1).

The enzyme has been evaluated for its safety and technological need and is authorised by Denmark and France.

### ***The advantages of the proposed change over the status quo***

Since the 1970s proteases have been increasingly used in various industrial food applications for hydrolysis of proteins. Protein hydrolysates can also be produced by acid and alkaline hydrolysis as well as by heat treatment.

As compared with these alternatives, the benefits of the action of serine protease are:

- Higher yield of soluble proteins and peptides
- Mild process conditions
- Reduced amounts of salts compared to acid hydrolysed protein
- Protein hydrolysate with controlled peptide profile due to specificity of the enzyme
- Increased digestibility of proteins

The serine protease object to this dossier has preferential cleavage at tyrosine, phenylalanine, leucine and methionine.

The resulting peptides are used as ingredients in a variety of food products. The applicability of use as food ingredients are often determined by the functional properties of the processed proteins which to a large extent are governed by their molecular size and their distribution of hydrophobic amino acids. Enzymatic processing of proteins using selected proteases to hydrolyse specific peptide bonds is widely used to produce peptides with e.g. increased solubility, modified viscosity and altered foaming, gelling and emulsifying properties. Processing of proteins is also used to improve the digestibility of proteins and thereby the nutritional value of the protein source.

Examples of current applications are protein fortification, seeking improved functionality and flavour of hydrolysed protein in dietary drinks, dry blended beverages and nutritional bars, and infant food, seeking improved digestibility of vegetable based formulas and reduced allergenicity of cow milk based formulas. Further, proteases may be used for production of yeast extracts and for production of hydrolysed vegetable proteins to be further used in savoury snacks, soups and bouillon cubes, where especially flavour formation and lower salt content is of interest.

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Use of enzymes in protein modification has been summarized by Nielsen P.M (2010<sup>7</sup>). In conclusion the use of proteases for modification of protein properties to produce protein ingredients with improved properties is well-established in the market. The expansion of the market with new innovative hydrolysed protein products is to a large extent dependent on the availability of enzymes. It is evident that controlling the properties of the peptides is a very complex task and may require a large palette of enzymes covering a range of different specificities. As stated above, none of the currently approved proteases in Australia have chymotrypsin specificity, which makes CTL3 conc BG an interesting option.

As a response to international customer interests, registration activities have been done or are in progress or planned, globally. The serine protease preparation is approved in Denmark and France and is going to be approved in Brazil and Mexico with the next update of the positive list. Furthermore, the enzyme preparation has been positively evaluated by JECFA.

An Australian customer support letter is attached as Appendix 1.1.

#### **A. Regulatory impact information**

The application is not likely to place costs or regulatory restrictions on industry or consumers. Inclusion of the serine protease enzyme in Standard 1.3.3 will provide the food industry with a microbial chymotrypsin solution giving potential customers the opportunity to produce protein hydrolysates with the desired functional properties. For government, the burden is limited to necessary activities for a variation of Standard 1.3.3.

### **3.1.5 Information to support the application**

#### ***Public health and safety issues related to the proposed change***

No public health and safety issues related to the proposed change are foreseen. As outlined in sections D, E, F, the serine protease is produced by submerged fermentation of a genetically modified *Bacillus licheniformis* strain.

- The enzyme preparation complies with international specifications ensuring absence of contamination by toxic substances or noxious microorganisms.
- The production organism has a long history of safe use as production strain for food grade enzyme preparations and is not known to produce any harmful metabolites.
- The genetic modifications in the production strain are well-characterized and safe and the integrated DNA (enzyme gene) has been shown to be stably maintained.
- Sequence homology assessment to known allergens and toxins shows that oral intake of the serine protease does not pose any food allergenic or toxic concern.
- Two mutagenicity studies show that the food enzyme is unable to damage the genetic material of living organisms.
- An oral toxicological study in rodents (a 13-weeks study), where groups of animals were given the food enzyme at very high doses, show, that all dose levels were generally well tolerated.

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### ***Consumer choice issues related to the proposed change***

No consumer choice issues related to the proposed change are foreseen. The enzyme is to be used as processing aid for partial or extensive hydrolysis of animal and vegetable proteins (such as casein, whey, gluten, and proteins from soy, corn, rice, peas, lentils, meat and fish) to be further used as ingredients in a variety of beverage and food products.

### ***Evidence that the food industry generally or other specific companies have an interest in, or support, the proposed change to the Code.***

The support letter from an Australian customer is attached as Appendix 1.1.

### **3.1.6 Assessment procedure**

Because the application is for a protease variant and different proteases already have been included in the Code, it is considered appropriate that the assessment procedure is characterized as “General Procedure, Level 1”.

### **3.1.7 Confidential commercial information (CCI)**

Detailed information on the construction and characteristics of the genetically modified production strain is provided in Appendix 6. A summary of this information is given in section E. The formal request for treatment of selected elements in Appendix 6 as commercial information (CCI) is included as Appendix 1.2.

### **3.1.8 Exclusive capturable commercial benefit (ECCB)**

This application is not expected to confer an Exclusive Capturable Commercial Benefit.

### **3.1.9 International and other national standards**

#### ***A. International Standards***

Use of serine protease as processing aid for hydrolysis of proteins during processing of protein containing food and food ingredients is not restricted by any Codex Alimentarius Commission (Codex) Standards.

#### ***B. Other national standards or regulations***

Use of serine protease as processing aid for hydrolysis of proteins during processing of protein containing food and food ingredients is not generally restricted by national standards or regulations.

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### **3.1.10 Statutory declaration**

The Statutory Declaration is included as Appendix 1.3.

### **3.1.11 Checklist**

This application concerns an enzyme product intended to be used as a processing aid. Therefore, the relevant documentation according to the Application Handbook from Food Standards Australia New Zealand as of September 1<sup>st</sup> 2013, are the following sections:

- SECTION 3.1 – GENERAL REQUIREMENTS
- SECTION 3.3.2 – PROCESSING AIDS, subsections A, C, D, E, F

Accordingly, the checklist for General Requirements as well as the Processing Aids part of the checklist for Standards related to Substances added to Food was used and is included as Appendix 1.4 and 1.5.

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## SECTION 3.3, STANDARDS RELATED TO SUBSTANCES ADDED TO FOOD

### 3.3.2 PROCESSING AIDS

The serine protease enzyme preparation described in this application is representative of the commercial food enzyme product, CTL3 conc BG, on which approval is sought.

#### A. Technical information on the processing aid

##### A.1. Information on the type of processing aid

###### A.1.1. Type of processing aid

The serine protease enzyme preparation belongs to the category of processing aids described in Clause 17 of Standard 1.3.3., Enzymes of microbial origin.

The serine protease enzyme preparation is used for hydrolysis of proteins during processing of protein containing foods and food ingredients

The active enzyme is a serine protease (EC 3.4.21.1) that catalyses the hydrolysis of peptide bonds in proteins with preferential cleavage at tyrosine phenylalanine, leucine and methionine. The reaction products are smaller proteins and peptides of variable lengths.

The enzyme is used for partial or extensive hydrolysis of animal and vegetable proteins such as casein, whey, gluten, and proteins from soy, corn, rice, peas, lentils, meat and fish.

In principle, the enzymatic conversion of proteins with the help of serine protease can be of benefit in the processing of all foods and food ingredients which naturally contain the substrate.

The maximum recommended dosage for production of solid and liquid food is 600 KMCU per kg protein raw material.

###### A.1.2 Evidence that the form and the amount of the processing aid performs the intended function.

CTL3 conc BG is useful when a moderate, controlled hydrolysis is desirable. Such moderate hydrolysis is well known to improve general functionality e.g. emulsifying capacity, solubility, foaming etc. compared to the native protein (Gauthier and Pouliot, 2003<sup>1</sup> and van der Ven et al, 2002<sup>2</sup>).

Figure 1 shows the effect of CTL3 conc BG on milk protein (5% whey concentrate) when added in a dosage of 600 KMCU/kg protein. The hydrolysis reaction was performed as a function of time (T=120 min), at 52 °C and pH 7.5. The Degree of Hydrolysis (%DH) obtained was around 9.5. On average basis a %DH in the range of 6-10 is well known to result in the above mentioned functionalities.

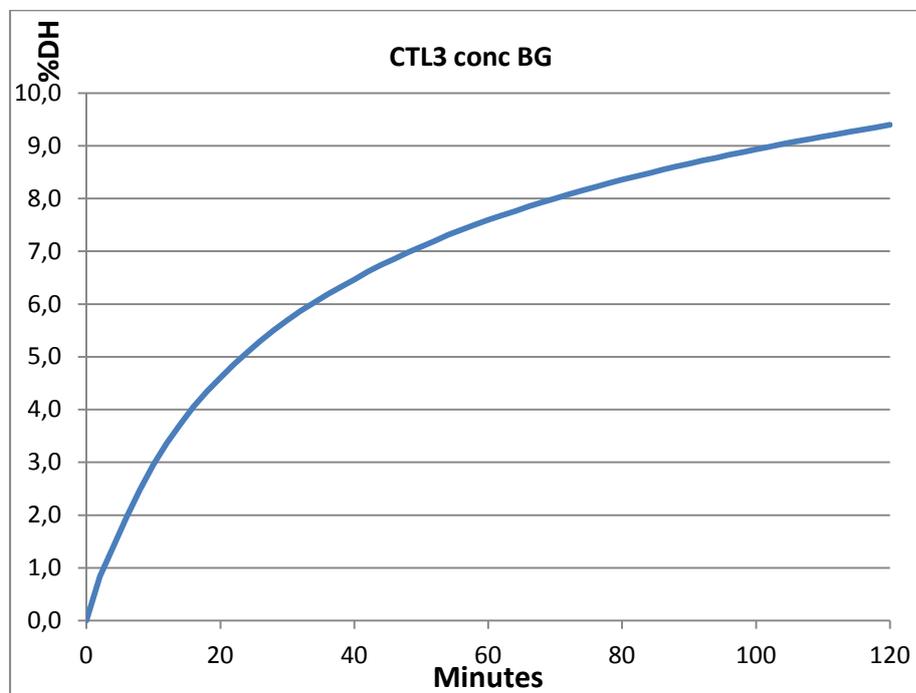


Fig 1. Degree of hydrolysis (%DH) as a function of time (T= 0-120 min).  
Enzyme dosage: 600 KMCU/kg protein; substrate: 5 % whey concentrate; pH 7.5; temp. 52°C..

## A.2. Information on the identity of the processing aid

### A.2.1. Enzyme

Generic name:	Serine protease
IUBMB nomenclature:	Chymotrypsin
IUBMB No.:	EC 3.4.21.1
CAS No.:	9004-07-3

### A.2.2. Enzyme preparation

Commercial name: CTL3 conc BG

The serine protease enzyme preparation is available as a single enzyme granulate stabilized with sucrose.

The Product Data Sheet for CTL3 conc BG is enclosed as Appendix 2.1.

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The typical composition of CTL3 conc BG is shown below:

	<u>CTL3 conc BG</u>
Enzyme solids (TOS <sup>a</sup> )	Approx. 85%
Sucrose	approx. 10%
Water	approx. 5%

CTL3 conc BG is produced with an activity of approx. 650 KMCU/g. The Novozymes method used to determine the KMCU activity is enclosed in Appendix 3.1.

The serine protease hydrolyses the substrate Suc-Ala-Ala-Pro-Phe-pNA. The release of yellow pNA results in an increase in absorbance at 405 nm, and this increase is proportional to the enzyme activity.

#### *A.2.3. Host organism*

The host strain is a modified (non-sporulating, protease deficient) *Bacillus licheniformis* strain derived from a natural isolate of *B. licheniformis*, DSM 9552 (equals to ATCC 9789).

The following comprises the taxonomy of the host strain:

Name: *Bacillus licheniformis*  
Class: Bacilli  
Order: *Bacillales*  
Genus: *Bacillus*  
Species: *licheniformis*

For a more detailed description of the host organism and the genetic modifications, please see section E.

#### *A.2.4. Donor organism*

The serine protease is derived from *Nocardioopsis prasina*.

For a more detailed description of the donor and the donor gene as well as donor for promoter and terminator, please see section E.

### ***A.3. Information on the chemical and physical properties of the processing aid***

The active enzyme is a serine protease (EC 3.4.21.1) used for the hydrolysis of proteins during processing of protein containing foods and food ingredients.

No reaction products, which could not be considered normal constituents of the diet, are formed during the production or storage of the enzyme treated food.

CTL3 conc BG is available as high concentrated granulates stabilized with sucrose.

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<sup>a</sup> TOS = Total Organic Solids, defined as: 100% - water - ash - diluents

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The protease is used as a processing aid in the manufacture of foods and food ingredients. The enzyme is denatured by high temperatures used in the manufacturing process and does not have any action or any function in the final food product.

#### **A.4. Manufacturing process**

The manufacturing process is composed of a fermentation process, a purification process, a formulation process and finally a quality control of the finished product, as outlined by Aunstrup et al. 1979<sup>3</sup>.

This section describes the processes used in manufacturing of the serine protease enzyme product.

The enzyme preparation is manufactured in accordance with current Good Manufacturing Practices, Food (Appendix 4.1). The quality management system used in the manufacturing process complies with ISO 9001:2008 (Appendix 4.2).

The raw materials are Food Grade Quality and have been subjected to appropriate analysis to ensure their conformity with the specifications.

##### *A.4.1. Fermentation*

The serine protease is produced by submerged fed-batch pure culture fermentation of the genetically modified strain of *Bacillus licheniformis*, described in section E.

##### *A.4.1.1. Raw materials for fermentation*

The production strain is grown in a medium consisting of compounds providing an adequate supply of carbon and nitrogen and other nutrients necessary for growth. The choice of raw materials used in the fermentation process (the feed, the seed fermenter, the main fermenter and dosing) is listed below.

Potable water

Carbohydrates: (e.g. sucrose, glucose, maltose, starch hydrolysates)

Vegetable protein: (e.g. potato protein, ammonia, soy bean meal)

Salts: (e.g. magnesium sulphate, potassium hydroxide, phosphoric acid, calcium carbonate, sulphuric acid)

Other nutrients: Trace metals (e.g. MnSO<sub>4</sub>, FeSO<sub>4</sub>, CuSO<sub>4</sub>, ZnSO<sub>4</sub>)

pH adjustment agents: (e.g. citric acid, phosphoric acid, sodium hydroxide, ammonia)

Antifoaming agents: (if necessary, e.g. polypropylene glycol, modified polyalkoxyether)

##### *A.4.1.2. Hygienic precautions*

All equipment is designed and constructed to prevent contamination by foreign micro-organisms.

All valves and connections not in use for the fermentation are sealed by steam at more than 120°C.

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After sterilization a positive pressure of more than 0.2 atmosphere is maintained in the fermentation tank.

The air used for aeration is sterilized by passing a sterile filter.

The inside of each fermentation tank is cleaned between fermentations by means of a high-pressure water jet and inspected after the cleaning procedures have been completed.

#### *A.4.1.3. Preparation of the inoculum*

The inoculum flask containing the prepared medium is autoclaved and checked. Only approved flasks are used for inoculation.

The stock culture suspension is injected aseptically into the inoculum flask and spread onto the medium in the flask. Once growth has taken place in the inoculum flask (typically after a few days at 30°C), the following operations are performed:

- Strain identity and traceability: ampoule number is registered
- Microbial purity: a sample from the inoculum flask is controlled microscopically for absence of microbial contaminants.

When sufficient amount of biomass is obtained and when the microbiological analyses are approved, the inoculum flask can be used for inoculating the seed fermentor.

#### *A.4.1.4. The seed fermentation*

The raw materials for the fermentation medium are mixed with water in a mixing tank. The medium is transferred to the seed fermenter and heat sterilized (e.g. 120°C / 60 min).

The seed fermentation tank is inoculated by transferring aseptically a suspension of cells from the inoculum flask.

The seed fermentation is run aerobically (sterile airflow), under agitation. The overpressure is kept above 0.2 atmosphere at all times, to prevent contamination.

Once a sufficient amount of biomass has developed, microbiological analyses are performed to ensure absence of contamination. The seed fermentation can then be transferred to the main fermentation tank.

#### *A.4.1.5. The main fermentation*

The raw materials for the medium are mixed with water in a mixing tank. The medium is transferred to the main fermenter and heat sterilized (e.g. 120°C / 60 min). If necessary, the pH is adjusted after sterilization, with sterile pH adjustment solutions.

The fermentation in the main tank is run as normal submerged fed-batch fermentation.

The main fermentation is run aerobically (sterile airflow), under vigorous agitation. The overpressure is kept above 0.2 atmosphere at all times, to prevent contamination. The fermentation is run at a well-defined temperature.

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Fresh medium is added aseptically when the pH increases above its set point, and the dissolved oxygen concentration rises. The feed rate is adjusted so that there is no accumulation of carbohydrates.

Other parameters are measured at regular intervals

- Refractive index
- Enzyme productivity
- Residual glucose
- Residual ammonia.

Samples are also taken at regular intervals to check absence of microbial contamination.

#### *A.4.2. Recovery*

The recovery process is a multi-step operation designed to separate the enzyme from the microbial biomass and partially purify, concentrate, and stabilize the food enzyme.

The steps of this process involve a series of typical unit operations:

- Pre-treatment
- Primary separation
- Concentration
- Pre and germ filtration
- Stabilization
- Final concentration and granulation

##### *A.4.2.1. Raw materials for recovery*

The raw materials typically used in the recovery process are as follows:

Potable water

Filter aids: Diatomite or Perlite

Acids and bases for pH adjustment: Phosphoric acid, sodium hydroxide

Flocculants (e.g. Polymer of dimethylamin and epichlorhydrin, anionic polyacrylamide and poly(aluminium hydroxy)chloride)

##### *A.4.2.2. Pre-treatment*

To facilitate the separation, flocculants are used in a pH-controlled process.

##### *A.4.2.3. Primary separation*

The cell mass and other solids are separated from the broth by well-established techniques such as pre-coat vacuum drum filtration or centrifugation. The precoat used in the filter and the filter aid used in the process is diatomaceous earth (diatomite or perlite).

The primary separation is performed at well-defined pH and temperature range.

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#### A.4.2.4. *Concentration*

Ultrafiltration is applied for concentration and further purification. The ultrafiltration is applied to fractionate high molecular weight components (enzymes) from low molecular weight components and is used to increase the activity/dry matter ratio.

The pH and temperature are controlled during the concentration step, which is performed until the desired activity and activity/dry matter ratio has been obtained.

#### A.4.2.5. *Pre and germ filtration*

For removal of residual cells of the production strain and as a general precaution against microbial degradation, filtration on dedicated germ filtration media is applied. Pre-filtration is included when needed.

The filtrations are performed at well-defined pH and temperature intervals, and result in an enzyme concentrate solution free of the production strain and insoluble substrate components from the fermentation.

#### A.4.2.6. *Stabilization*

For physical stabilization sucrose is added to the enzyme concentrate.

#### A.4.2.7. *Final Concentration and Granulation*

Final concentration is carried out by evaporation and/or ultra filtration. Finally, the product is spray dried.

#### A.4.2.8. *Process control*

Apart from the process controls performed during the various fermentation steps and described above, the following microbial controls are also performed.

Samples are withdrawn from both the seed fermenter and the main fermenter:

- a) before inoculation
- b) at regular interval during cultivation
- c) before transfer/harvest

The samples during all steps are examined by:

- a) microscopy
- b) plating culture broth on a nutrient agar and incubating for 24-48 hours.

Growth characteristics are observed macroscopically and microscopically.

During the microbiological control steps, the number of foreign micro-organisms should be insignificant. The fermentation parameters, i.e. enzyme activity, temperature and oxygen as well as pH are also monitored closely. A deviation from the normal course of the fermentation may signal a contamination.

If a significant contamination develops, the fermentation is terminated. The fermentation is regarded as “significantly contaminated” if two independent samples show presence of contaminating organisms after growth on nutrient agar.

Any contaminated fermentation is rejected for enzyme preparations to be used in a food grade application.

#### **A.5. Specification for identity and purity**

The serine protease enzyme product complies with the purity criteria recommended for Enzyme Preparations in Food, Food Chemical Codex, 8th edition, 2012.

In addition to this, the serine protease enzyme product also conforms to the General Specifications for Enzyme Preparations Used in Food Processing as proposed by the Joint FAO/WHO Expert Committee on Food Additives in Compendium of Food Additive Specifications, available online at: <http://www.fao.org/ag/agn/jecfa-additives/search.html?lang=en>

Analytical data for an unstandardized representative serine protease batch is shown in the table below. These data show compliance with the purity criteria of the specification.

Control parameter	Unit	Specification	Batch PPA 26797
Serine protease activity	KMCU/g		54.6
Heavy Metals	ppm	Max 30	3.4
Pb	ppm	Max 5	ND (DL < 0.5)
As	ppm	Max 3	ND (DL < 0.1)
Cd	ppm	Max 0.5	ND (DL < 0.05)
Hg	ppm	Max 0.5	ND (DL < 0.03)
Total viable count	/g	Not more than 50000	< 200
Total coliforms	/g	Not more than 30	< 10
Enteropathogenic E. coli	/25g	Not detected	ND
Salmonella	/25g	Not detected	ND
Antibiotic activity		Not detected	ND
Production strain	/g	Not detected	ND

Heavy Metals =  $\Sigma$  of Ag, As, Bi, Cd, Cu, Hg, Mo, Ni, Pb, Sb, Sn

ND = Not Detected

DL = Detection limit

The methods of analysis used to determine compliance with the specifications are enclosed in Appendix 3.

The Product Data Sheet for CTL3 conc BG is enclosed in Appendix 2.1.

The typical composition is shown below:

	<u>CTL3 conc BG</u>
Enzyme solids (TOS <sup>b</sup> )	approx. 85 %
Sucrose	approx. 10 %
Water	approx. 5 %

<sup>b</sup> TOS = Total Organic Solids, defined as: 100% - water - ash - diluents

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CTL3 conc BG is produced as a highly concentrated granulated product that is stabilized with sucrose. It is standardized to a product strength of above 650 KMCU/g. The Novozymes method used to determine the KMCU activity is enclosed in Appendix 3.1.

CTL3 conc BG does not contain known food allergens as detailed in the Product Data Sheet in Appendix 2.1.

The serine protease is not present as particulate matter in the final food as the enzyme is being subjected to a heat denaturation step once the desired degree of hydrolysis is obtained in the protein hydrolysis process.

#### **A.6. Analytical method for detection**

The serine protease enzyme preparation is to be used in the food industry as a processing aid. This information is not required in the case of an enzymatic processing aid.

### **B. Information related to the safety of a chemical processing aid**

Not applicable – this application does not concern a chemical processing aid.

### **C. Information related to the safety of an enzyme processing aid**

#### **C.1. General information on the use of the enzyme as a food processing aid in other countries**

The serine protease is used as a processing aid for hydrolysis of proteins during processing of protein containing food and food ingredients.

Dossiers have been submitted to relevant authorities and expert bodies for evaluation of safety and technological need.

The regulatory status for the serine protease object of this dossier is as follows:

- Denmark: The enzyme preparation has been safety assessed according to the Guidelines for the evaluation of food enzymes (the Scientific Committee for Food, Commission of the European Communities, 1992<sup>4</sup>). This resulted in the authorisation of the enzyme product by the Danish authorities.
- France: The enzyme has been positively evaluated by the French Authorities and has been included in The French order of October 19, 2006 on use of processing aids in the manufacture of certain foodstuff, as amended
- JECFA: The enzyme preparation has been positively evaluated in the 76th meeting of JECFA and has been allocated an Acceptable Daily Intake (ADI) “not specified”.
- Mexico: The enzyme has been positively evaluated by COFEPRIS, however the amendment to the positive list is awaiting the next official update.

- 
- Brazil: The enzyme has furthermore been positively evaluated by ANVISA, however the amendment to the positive list is awaiting the next official update, expected in 2014.

## **C.2. Information on the potential toxicity of the enzyme processing aid**

### *(a) Information on the enzyme's prior history of human consumption and/or its similarity to proteins with a history of safe human consumption*

A wide variety of enzymes, including proteases, are used in food processing and have a long history of safe use in food (Pariza and Foster, 1983<sup>5</sup>; Pariza and Johnson, 2001<sup>6</sup>).

Enzymatic processing of proteins using selected proteases to hydrolyse specific peptide bonds has been used since the 1970s to produce peptides with improved functional properties (Nielsen, 2010<sup>7</sup>).

Proteases from various micro-organisms including *Bacillus licheniformis* are widely accepted by authorities to be used as processing aids in various food applications.

The Food and Drug Administration has affirmed that mixed carbohydrase and protease enzyme products derived from *Bacillus licheniformis* are generally recognised as safe (GRAS) in the production of certain foods including nutritive sweeteners, see 21CFR §184.1027 (FDA, 1983<sup>8</sup>).

JECFA has evaluated the serine protease object of the present dossier and concluded that this food enzyme does not constitute a toxicological hazard (JECFA, 2012<sup>9,10,11</sup>).

### *(b) Information on any significant similarity between the amino acid sequence of the enzyme and that of known protein toxins*

A sequence homology assessment of the serine protease enzyme to known toxins and allergens was conducted. No homologies to toxins or allergens were found. The complete search report is enclosed in Appendix 5.1.

Furthermore, safety studies as described below were performed on a representative batch (PPA 26797) that was produced according to the description given in section A.4, omitting stabilization and standardization.

The following studies were performed:

A summary of the safety studies is enclosed in Appendix 5.2.

- Ames Test. Test for mutagenic activity (Appendix 5.3)
- In vitro chromosome aberration test (Appendix 5.4)
- Subchronic (13 week) oral toxicity study in rats (Appendix 5.5)

The main conclusions of the safety studies can be summarized as follows:

- Serine protease, PPA 26797 did not induce gene mutations in the Ames test, neither in the presence or absence of S-9 mix.
- Serine protease, PPA 26797 did not induce chromosome aberrations in cultured human blood lymphocytes when tested up to 5000 µg/mL in the presence and absence of S-9 mix.

- 
- Thirteen weeks of oral administration (by gavage) of serine protease PPA 26797 at dose levels up to 500.1 mg Total Organic Solids (TOS) per kg body weight (bw) per day resulted in no treatment-related effects. In this study the no observed adverse effect level (NOAEL) in rats treated orally by gavage for 13 weeks was considered to be the highest dose level administered, equivalent to 500.1 mg Total Organic Solids (TOS) per kg bw/day or 287.5 KMCU<sup>c</sup> /kg bw/day.

Based on the present toxicity data it can be concluded that the serine protease enzyme preparation, represented by batch PPA 26797, exhibits no toxicological effects under the experimental conditions described.

### **C.3. Information on the potential allergenicity of the enzyme processing aid**

#### *(a) Information of the source of the enzyme processing aid*

The serine protease enzyme is produced by a *Bacillus licheniformis* microorganism expressing serine protease from *Nocardiopsis prasina*.

*Bacillus licheniformis* is a soil and plant living saprophyte, recognized as non-pathogenic species for humans, animals and plants (see Section D).

#### *(b) Analysis of similarity between the amino acid sequence of the enzyme and that of known allergens*

Enzymes have a long history of safe use in food, with no indication of adverse effects or reactions. Moreover a wide variety of enzyme classes (and structures) are naturally present in food.

The allergenicity potential of enzymes was studied by Bindslev-Jensen et al (2006<sup>12</sup>) and reported in the publication: "Investigation on possible allergenicity of 19 different commercial enzymes used in the food industry". The investigation comprised enzymes produced by wild-type and genetically modified strains as well as wild-type enzymes and protein engineered variants and comprised 400 patients with a diagnosed allergy to inhalation allergens, food allergens, bee or wasp. It was concluded from this study that ingestion of food enzymes in general is not likely to be a concern with regard to food allergy.

Additionally, food enzyme are used in small amounts during food processing resulting in very small amounts of the enzyme protein in the final food. A high concentration generally equals a higher risk of sensitization, whereas a low level in the final food equals a lower risk (Goodman et al, 2008<sup>13</sup>).

A sequence homology assessment of the protease enzyme to known toxins and allergens was conducted (Appendix 5.1). No homologies to toxins or allergens were found.

Consequently, oral intake of the serine protease is not anticipated to pose any food allergenic concern.

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<sup>c</sup> See Appendix 3.2.

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#### **C.4. Safety assessment reports prepared by international agencies or other national government agencies, if available**

A document certifying approval of the serine protease object of this dossier by the Danish authorities is enclosed as Appendix 2.2. The safety evaluation was done according to the Guidelines for food enzymes by the Scientific Committee for Food (Appendix 2.3).

The Danish approval, which was given back in 2010, was based on a liquid product variant of the same enzyme product. At that time, the enzyme activity was standardized in a different activity unit<sup>d</sup>. The commercial name for the liquid product variant was iZyme B.

In addition, JECFA has evaluated the serine protease object of the present dossier and concluded that this food enzyme does not constitute a toxicological hazard (JECFA, 2012<sup>9,10,11</sup>).

### **D. Additional information related to the safety of an enzyme processing aid derived from a microorganism**

#### **D.1. Information on the source microorganism**

The serine protease enzyme is produced by a *Bacillus licheniformis* microorganism expressing serine protease from *Nocardiopsis prasina*. The host strain is a modified (non-sporulating, protease deficient) *Bacillus licheniformis* strain derived from a natural isolate of *B. licheniformis*, DSM 9552 (equals to ATCC 9789).

#### **D.2. Information on the pathogenicity and toxicity of the source microorganism**

*Bacillus licheniformis* is a soil and plant living saprophyte, recognized as non-pathogenic species for humans, animals and plants (Priest FG, 1993<sup>14</sup>, de Boer AS *et al*, 1994<sup>15</sup>, EPA, 1997<sup>16</sup>). *B. licheniformis* is also common in foods including natural agricultural products such as cereals.

*B. licheniformis* is classified as a group 1 microorganism according to EU Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work. A group 1 biological agent means one that is unlikely to cause human disease.

The host strain is sporulation deficient.

Industrial strains belonging to the *B. licheniformis* species have a long history of safe use in food enzyme manufacturing. They have been used for decades in the production of enzymes, and in more than a decade as recombinant organisms for the production of a variety of bio-industrial products like food grade enzymes, vitamins, antibiotics, and additives (Schallmeyer M *et al*, 2004<sup>17</sup>).

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<sup>d</sup> See Appendix 3.2

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The Food and Drug Administration has affirmed that mixed carbohydrase and protease enzyme products derived from *B. licheniformis* are generally recognized as safe (GRAS) in the production of certain foods including nutritive sweeteners, see 21CFR §184.1027. In the supplementary information to the final rule in the Federal Register, FDA emphasized that "Published scientific literature as well as standard books on food microbiology demonstrate that *B. licheniformis* is widely recognized as a common contaminant found in many foods. None of these references report any toxicity or pathogenicity associated with the presence of this organism in food."

In addition, the FDA did not question the conclusion that various other food enzymes obtained from genetically modified *B. licheniformis* strains are GRAS under the intended conditions of use (GRN no. 22, GRN no. 24, GRN no. 72, GRN no. 79, GRN no. 265, GRN no. 277).

JECFA has evaluated enzymes derived from *B. licheniformis*, including the serine protease object of this dossier<sup>9,10,11</sup>, and concluded that these food enzymes do not constitute a toxicological hazard.

The non-pathogenicity and non-toxicity of *B. licheniformis* is thus strongly supported by the historic record of this organism.

### ***D.3. Information on the genetic stability of the source organism***

The inserted recombinant DNA is genetically stable during fermentation, as the inserted DNA is integrated into the chromosome.

The genetic stability of the production strain was tested at large-scale fermentation. The strain stability during fermentation was analyzed by Southern blotting. No instability of the strain was observed (Appendix 6.5).

For a more detailed description of the strain construction and characteristics, please see section E.

## **E. Additional information related to the safety of an enzyme processing aid derived from a genetically-modified microorganism**

### ***E.1. Information on the methods used in the genetic modification of the source organism***

This section contains summarized information on the modifications of the host strain, on the content and nature of the introduced DNA and on the construction of the final production strain, as well as the stability of the inserted gene. The detailed information is provided in Appendix 6.

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### E.1.1. Host organism

The parental strain Ca63 is a natural isolate and the taxonomic classification is as followed:

Name:	<i>Bacillus licheniformis</i>
Phylum:	Firmicutes
Class:	Bacilli
Order:	<i>Bacillales</i>
Genus:	<i>Bacillus</i>
Species:	<i>licheniformis</i>

The classification was confirmed by Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH.

The parental strain Ca63 was subjected to a single round of classical mutagenesis leading to a sporulation deficient variant Si3.

### Genetic modifications

Si3 was further modified at several chromosomal loci. The resulting host strain SJ6370 has deletions in two endoprotease genes leading to an abolished production of these proteins. The modifications were done to obtain increased enzyme yield and increased enzyme purity. Additionally the *amyL* locus and *xyIA* locus were modified in order to facilitate integration and expression of the serine protease genes.

### E.1.2. Introduced DNA

The serine protease gene construct consists of DNA sequences encoding the *Nocardiopsis prasina* serine protease pro+ mature regions.

The promoter is a triple promoter composed of promoter elements from three different *Bacillus* donors. The transcriptional terminator is from the *Bacillus licheniformis* alpha-amylase gene. Well-known plasmids were used for vector constructs.

The inserted expression cassettes, expressing the serine protease were integrated into the production strain by double homologous recombination at two different chromosomal locations, the *amyL* region and the *xyIA* region. The expression cassette itself contains two open reading frames (genes) in tandem, having slightly different DNA sequences but both encoding the same protein. This means that there are a total of 4 open reading frames (genes) expressing the serine protease in the final production strain.

The full DNA sequences of the introduced DNA present in the production strain are given in Appendix 6.

### E.1.3. Construction of the Recombinant Microorganism

#### Introduction of the serine protease genes at the *amyL* locus:

The segment encoding a *Bacillus* mannanase was replaced with a DNA segment containing a tandem gene construct encoding the *Nocardiopsis prasina* serine protease. The two genes encoding the serine protease are consequently expressed from the triple promoter, present at the chromosomal *amyL* locus.

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### Introduction of serine protease genes at the *xyIA* locus:

The DNA segment containing a tandem gene construct encoding the *Nocardioopsis prasina* serine protease was inserted so that it is expressed from the triple promoter present at the *xyIA* position in the chromosome.

### Abolishment of background protein

Further, the ribosome binding site (RBS) in front of a background protein was replaced with a non-functional RBS version leading to an abolished production of this protein.

### Description of the production organism

The chromosome of the final production strain has been modified by recombinant DNA techniques at five different positions relative to the non-recombinant, non-sporulating strain Si3. These positions are

- A) The position of the gene encoding an alkaline protease where a deletion was introduced.
- B) The position of the gene encoding a glu-specific protease, where a deletion was introduced.
- C) The position of the gene encoding a background protein, where the ribosome binding site was modified.
- D) The position of the gene encoding the alpha-amylase, *amyL*, where the expression cassette with the tandem serine protease gene construct was inserted.
- E) The position of the gene encoding the xylose isomerase, *xyIA*, where the expression cassette with the tandem serine protease gene construct was inserted

The resulting strain was subsequently subjected to classical mutagenesis and a production strain giving a high yield was selected.

#### *E.1.4. Antibiotic Resistance Gene*

No functional antibiotic resistance genes were left in the strain as a result of the genetic modifications. The absence of antibiotic resistance genes was verified by Southern blot analysis using the relevant antibiotic resistance gene probes.

#### *E.1.5. Stability of the Introduced Genetic Sequences*

The presence of the introduced DNA sequences was also determined by Southern hybridization to assess the stability and potential for transfer of genetic material as a component of the safety evaluation of the production microorganism (Appendix 6).

The transforming DNA is stably integrated into the *B. licheniformis* chromosome and, as such, is poorly mobilizable for genetic transfer to other organisms and is mitotically stable.

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## **F. Information related to the dietary exposure to the processing aid**

### ***F.1. A list of foods or food groups likely to contain the processing aid or its metabolites***

The serine protease in this application is able to be used in the processing of all food groups containing intact protein where the intact protein is to be hydrolysed.

The enzyme acts on both animal and vegetable proteins such as casein, whey, gluten, and proteins from soy, corn, rice, peas, lentils, meat and fish.

The enzyme is used in food manufacturing as a processing aid to ease and optimise the hydrolysis process and to get a high yield and controlled fraction of soluble proteins and peptides with different functional properties, e.g. increased solubility, modified viscosity, altered foaming, gelling and emulsifying properties or improved digestibility (Nielsen, 2010<sup>7</sup>).

The enzyme is denatured by high temperatures used in the manufacturing process and does not have any action or function in the final food product.

### ***F.2. The levels of residues of the processing aid or its metabolites for each food or food group***

The serine protease enzyme preparation is used at minimum levels necessary to achieve the desired effect and according to requirements for normal production following cGMP.

The serine protease exerts its activity during the protein hydrolysis step. The enzyme is denatured by heat at the termination step. No reaction products, which could not be considered normal constituents of the diet, are formed during the production or storage of the enzyme treated food.

#### *F.2.1. Estimates of human consumption*

An exposure assessment according to the Budget Method (Hansen, 1966<sup>18</sup>; Douglass et al., 1997<sup>19</sup>; ILSI, 1997<sup>20</sup>) has been performed, as the processed proteins are used as ingredients in a variety of beverage and food products.

#### Budget method

The Budget Method assumptions represent a "maximum worst case" situation of human consumption, in which the food enzyme object of the present dossier would be used at its maximum recommended dosages in all processed food and all processed beverages.

The Budget Method also assumes that all of the food enzyme will end up in the final food. This assumption is exaggerated since the enzyme protein and the other substances resulting from the fermentation are diluted. Therefore, the safety margin calculation derived from this method is highly conservative.

## Assumptions in the Budget Method

<b>Solid food</b>	<p>The maximum energy intake over the course of a lifetime is 50 kcal/kg body weight/day. 50 kcal corresponds to 25 g food. Therefore, adults ingest 25 g food per kg body weight per day. Assuming that 50% of the food is processed food, the daily consumption of processed food will be 12.5 g processed foods per kg body weight. It is further assumed that, in average, all processed food contains 10% protein hydrolysate dry matter = 1.25 g protein hydrolysate dry matter per kg body weight per day.</p>
<b>Liquids</b>	<p>The maximum intake of liquids (other than milk) is 100 ml/kg body weight day. Assuming that 25% of the non-milk beverages is processed, the daily consumption will be 25 ml processed beverages per kg body weight. It is further assumed that all processed beverages contain 3.5% protein hydrolysate = 0.875 g protein hydrolysate dry matter per kg body weight per day. It is assumed that the densities of the beverages are ~ 1.</p>

### TMDI (Total amount of dietary intake)

The maximum recommended dosage of CTL3 conc BG for production of solid and liquid food is 600 KMCU per kg protein raw material.

A dosage of 600 KMCU per kg protein raw material corresponds to that 785 mg enzyme TOS (total organic solids) is transferred to one kg protein raw material.

The above activity/TOS relation is based on the typical composition data as given in the Product Data Sheet for CTL3 conc BG (Appendix 2.1).

- Declared activity: 650 KMCU/g enzyme product
- TOS content: 85g TOS/100g enzyme product

### TMDI solid food

Based on the given recommended dosage, 1.25 g protein hydrolysate dry matter in solid food will contain a maximum: 785 mg TOS per kg / 1000 g per kg x 1.25 g = 0.98 mg TOS

### TMDI liquids

Based on the given recommended dosage, 0.875 g protein hydrolysate dry matter in liquids will contain a maximum: 785 mg TOS per kg / 1000 g per kg x 0.875 g = 0.69 mg TOS

### TMDI solid food + liquids

The theoretical maximum daily intake (TMDI) of consumers of the food enzyme via various beverage and food products is: 0.98 + 0.69 = 1.67 mg TOS/kg body weight/day.

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### *F.2.2. Safety Margin Calculation*

The NOAEL in the 13 weeks oral toxicity study in rats was concluded to be the highest administered dose level, i.e. 500.1 mg TOS/kg bw/day (see section C.2. and Appendix 5.5). Based on the calculated theoretical "worst case" TMDI as described in the section above, a safety margin can be calculated as the NOAEL divided by the TMDI, as seen below.

NOAEL (mg TOS/kg bw/day)	500.1
TMDI (solid food + liquids) (mg TOS/kg bw/day)	1.67
Safety margin (Budget method)	<b>299</b>

### ***F.3. For foods or food groups not currently listed in the most recent Australian or New Zealand National Nutrition Surveys (NNSs), information on the likely level of consumption***

Not relevant. In the estimate on human consumption given in F.2.1 above, it is assumed that all food and beverages containing protein hydrolysates are produced using CTL3 conc BG as a processing aid at the highest recommended dosage.

### ***F.4. The percentage of the food group in which the processing aid is likely to be found or the percentage of the market likely to use the processing aid***

In the estimate on human consumption given in F.2.1 above, it is assumed that all food and beverages containing protein hydrolysates are produced using CTL3 conc BG as a processing aid at the highest recommended dosage.

### ***F.5. Information relating to the levels of residues in foods in other countries***

For the estimate on human consumption given in F.2.1 above, consumption data from UK, DK and US were used.

### ***F.6. For foods where consumption has changed in recent years, information on likely current food consumption***

For the estimate on human consumption given in F.2.1 the Budget Method has been used representing a "maximum worst case" situation of human consumption.

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## List of Appendices

- 1) General Requirements
- 2) Product information
- 3) Methods of analysis used to determine compliance with the specifications
- 4) Documentation regarding the manufacturing process
- 5) Safety documentation
- 6) Documentation regarding the production microorganism

# Appendix 1

## General Requirements

1. Evidence that the food industry generally or other specific companies have an interest in, or support, the proposed change to the Code
2. Formal request for treatment of confidential commercial information (CCI)
3. Statutory declaration
4. Checklist for GENERAL REQUIREMENT
5. Checklist for Standards related to Substances added to Food

Nestlé Australia Ltd.

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[REDACTED]  
Novozymes A/S  
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1 July 2014

[REDACTED]  
The serine protease enzyme preparation, CTL3 conc BG, has been tested in our pilot plant with very good results.

CTL3 conc BG is useful when a moderate, controlled hydrolysis is desirable. The chymotrypsin specificity of the enzyme makes it an interesting alternative/ supplement to other proteases currently used by Nestlé for various food applications.

We would therefore highly support an application seeking approval of CTL-3 conc BG in Australia/New Zealand.

Please let us know if we can be of further assistance in the approval process of CTL3 conc BG.

Regards,

[REDACTED]  
Manager, Regulatory & Scientific Affairs Oceania  
Nestlé Australia Ltd

## Appendix 1.2

### Formal request for treatment of confidential commercial information (CCI)

Novozymes respectfully request that parts of Appendix 6 are treated as confidential commercial information (CCI).

The parts marked confidential in Appendix 6 contain detailed description of the construction of the genetically modified production strain and the introduced DNA. While individual steps in the DNA construction might be well known or publicly available information, the exact steps and sequence of those constitutes information that represent the state-of-the-art of one of Novozymes' core technologies, which has been obtained as a result of substantial investment in research and development within rDNA technology. Therefore, the marked information in Appendix 6 is claimed confidential for an unlimited period of time.

Furthermore, the sequence for serine protease provides an unambiguous possibility for our competitors to link a certain serine protease to a specific commercial product which would make it extremely easy for our competitors to copy it or benchmark their product against ours.

July 16<sup>th</sup> 2014



Senior Regulatory Specialist  
Regulatory Affairs  
Novozymes A/S

## Statutory Declaration – Australia

The information provided in Parts 1 to 3 must be attested to by a statutory declaration in some suitable form along the following lines:

### STATUTORY DECLARATION

**Re: Serine protease from Nocardioopsis prasina**

*Statutory Declarations Act 1959*<sup>1</sup>

I, Anthony James Bryan, 6 Belcote Road Longueville, NSW and director of Novozymes Australia Pty Ltd

make the following declaration under the *Statutory Declarations Act 1959*:

1. the information provided in this application fully sets out the matters required
2. the information provided in this application is true to the best of my knowledge and belief
3. no information has been withheld that might prejudice this application, to the best of my knowledge and belief

I understand that a person who intentionally makes a false statement in a statutory declaration is guilty of an offence under section 11 of the *Statutory Declarations Act 1959*, and I believe that the statements in this declaration are true in every particular.

[Signature of person making the declaration] 

Declared at North Rocks on 3<sup>rd</sup> of July 2014.

Before me,

  
NSW JP   
355 North Rocks Road  
North Rocks NSW 2151

[Signature of person before whom the declaration is made]<sup>2</sup>

[Full name, qualification and address of person before whom the declaration is made (in printed letters)]

I,   
a JP for NSW, certify:

1. I saw the face of the declarant/deponent and
2. ~~\*I have known the person for at least 12 mths OR~~  
\*I confirmed the person's identity with

NSW DRIVERS LICENCE 

  
3/7/2014  
Date

<sup>1</sup> <http://www.comlaw.gov.au/Series/C1959A00052>.

<sup>2</sup> A statutory declaration must be made before a prescribed person under the *Statutory Declarations Act 1959*. The list of prescribed persons is available in the *Statutory Declarations Regulations 1993* at <http://www.comlaw.gov.au/Series/F1996B00198>.

## Appendix 1.4

### Checklist for GENERAL REQUIREMENTS

#### General requirements (3.1)

- |  |   |
|--|---|
| <input checked="" type="checkbox"/> 3.1.1 Form of application <ul style="list-style-type: none"><li><input checked="" type="checkbox"/> <i>Application, abstracts and other key documents in English</i></li><li><input checked="" type="checkbox"/> <i>Executive Summary (separated from main application electronically and in hard copy)</i></li><li><input checked="" type="checkbox"/> <i>Relevant sections of Part 3 clearly identified</i></li><li><input checked="" type="checkbox"/> <i>Pages sequentially numbered</i></li><li><input checked="" type="checkbox"/> <i>Electronic copy (searchable)</i></li><li><input checked="" type="checkbox"/> <i>1 hard copy</i></li><li><input checked="" type="checkbox"/> <i>Electronic and hard copy identical</i></li><li><input checked="" type="checkbox"/> <i>Hard copy capable of being laid flat</i></li><li><input checked="" type="checkbox"/> <i>All references provided (in electronic and hard copy)</i></li></ul> | <input checked="" type="checkbox"/> 3.1.6 Assessment procedure <ul style="list-style-type: none"><li><input checked="" type="checkbox"/> <i>General</i></li><li><input type="checkbox"/> <i>Major</i></li><li><input type="checkbox"/> <i>Minor</i></li><li><input type="checkbox"/> <i>High level health claim variation</i></li></ul>   |
| <input checked="" type="checkbox"/> 3.1.2 Applicant details  | <input type="checkbox"/> 3.1.7 Confidential Commercial Information <ul style="list-style-type: none"><li><input checked="" type="checkbox"/> <i>Confidential material separated in both electronic and hard copy</i></li><li><input checked="" type="checkbox"/> <i>Formal request including reasons</i></li><li><input checked="" type="checkbox"/> <i>Non-confidential summary provided</i></li></ul> |
| <input checked="" type="checkbox"/> 3.1.3 Purpose of the application   | <input type="checkbox"/> 3.1.8 Exclusive Capturable Commercial Benefit <ul style="list-style-type: none"><li><input type="checkbox"/> <i>Justification provided</i></li></ul>   |
| <input checked="" type="checkbox"/> 3.1.4 Justification for the application <ul style="list-style-type: none"><li><input checked="" type="checkbox"/> <i>Regulatory impact information</i></li><li><input checked="" type="checkbox"/> <i>Impact on international trade</i></li></ul>  | <input checked="" type="checkbox"/> 3.1.9 International and other national standards <ul style="list-style-type: none"><li><input checked="" type="checkbox"/> <i>International standards</i></li><li><input checked="" type="checkbox"/> <i>Other national standards</i></li></ul>   |
| <input checked="" type="checkbox"/> 3.1.5 Information to support the application <ul style="list-style-type: none"><li><input checked="" type="checkbox"/> <i>Data requirements</i></li></ul>  | <input checked="" type="checkbox"/> 3.1.10 Statutory Declaration  |
|  | <input checked="" type="checkbox"/> 3.1.11 Checklist/s provided with application <ul style="list-style-type: none"><li><input checked="" type="checkbox"/> <i>3.1 Checklist</i></li><li><input checked="" type="checkbox"/> <i>Any other relevant checklists for Parts 3.2-3.7</i></li></ul>  |

## Appendix 1.5

### Checklist for Standards related to Substances added to Food

Processing Aids (3.3.2)	
<input checked="" type="checkbox"/> A.1 Type of processing aid	<input checked="" type="checkbox"/> C.3. Allergenicity information of enzyme (enzyme only)
<input checked="" type="checkbox"/> A.2 Identification information	<input checked="" type="checkbox"/> C.4. Overseas safety Assessment Reports
<input checked="" type="checkbox"/> A.3 Chemical and physical properties	<input checked="" type="checkbox"/> D.1 Information on source organism (enzyme from microorganism only)
<input checked="" type="checkbox"/> A.4 Manufacturing process	<input checked="" type="checkbox"/> D.2 Pathogenicity and toxicity of source microorganism (enzyme from microorganism only)
<input checked="" type="checkbox"/> A.5 Specification information	<input checked="" type="checkbox"/> D.3 Genetic stability of source organism (enzyme from microorganism only)
<input type="checkbox"/> A.6 Analytical method for detection	<input checked="" type="checkbox"/> E.1 Nature of genetic modification of source organism (enzyme from GM source microorganism)
<input type="checkbox"/> B.1 Industrial use information (chemical only)	<input checked="" type="checkbox"/> F.1 List of foods likely to contain the processing aid
<input type="checkbox"/> B.2 Information on use in other countries (chemical only)	<input checked="" type="checkbox"/> F.2 Anticipated residue levels in foods
<input type="checkbox"/> B.3 Toxicokinetics and metabolism information (chemical only)	<input type="checkbox"/> F.3 Information on likely level of consumption
<input type="checkbox"/> B.4 Toxicity information (chemical only)	<input checked="" type="checkbox"/> F.4 Percentage of food group to use processing aid
<input type="checkbox"/> B.5 Safety assessments from international agencies (chemical only)	<input checked="" type="checkbox"/> F.5 Information on residues in foods in other countries (if available)
<input checked="" type="checkbox"/> C.1 Information on enzyme use on other countries (enzyme only)	<input checked="" type="checkbox"/> F.6 Where consumption has changed, information on likely consumption
<input checked="" type="checkbox"/> C.2 Toxicity information of enzyme (enzyme only)	

## Appendix 2

### Product information

1. Product Data Sheet for CTL-3 conc BG
2. Certificate of approval for serine protease from *N. prasina* expressed in *B. licheniformis* by the Danish authorities. iZyme B (commercial product name referred to in approval certificate) is a liquid product variant of CTL-3 conc BG.
3. Statement from the Danish authorities regarding safety evaluation of new enzymes



## CTL3 conc BG

In this product the key enzyme activity is provided by endoprotease that hydrolyzes peptide bonds

### PRODUCT CHARACTERISTICS/PROPERTIES

Declared enzyme	Serine protease (Chymotrypsin)
Declared activity	
Color	Light brown
Physical form	Granulate
Particle size	Approx. 50-212 microns
<i>Color can vary from batch to batch. Color intensity is not an indication of enzyme activity.</i>	

### PRODUCT SPECIFICATION

	Lower Limit	Upper Limit	Unit
Protease unit KMCU	650		/g
Total viable count	-	10000	/g
Coliform bacteria	-	30	/g
E.coli	Not Detected		/25 g
Salmonella	Not Detected		/25 g
Heavy metals		Max 30	mg/kg
Lead		Max 5	mg/kg
Arsenic		Max 3	mg/kg
Cadmium		Max 0.5	mg/kg
Mercury		Max 0.5	mg/kg

The enzyme analytical method is available from the Customer Center or sales representative.

### COMPOSITION

Ingredients	Appr. % (w/w)
Serine protease (Chymotrypsin) CAS no. 9004-07-3*	85
Sucrose CAS no. 57-50-1	10
Water CAS no. 7732-18-5	5

\*Defined as enzyme conc. (dry matter basis)

### ALLERGEN

Allergen	Substance contained <sup>1</sup>	Allergen	Substance contained <sup>1</sup>
Beef	no	Lactose	no
Carrot	no	Legumes	no
Celery	no	Lupin	no
Cereals containing gluten <sup>2</sup>	no	Milk	no
Chicken meat	no	Molluscs	no
Cocoa	no	Mustard	no
Coriander	no	Nuts <sup>3</sup>	no
Corn/maize	no	Peanuts	no
Crustaceans	no	Pork	no
Egg	no	Sesame	no
Fish	no	Soy	no
Glutamate	no	Sulphur dioxide/sulphites more than 10 mg per kg or l	no

<sup>1</sup>Definition of substances according to LeDa/ALBA and EU Regulation 1169/2011 as amended

<sup>2</sup>i.e.wheat rye barley oats spelt kamut

<sup>3</sup>i.e. almond hazelnut walnut cashew pecan nut Brazil nut pistachio nut macadamia nut and Queensland nut

### NUTRITIONAL VALUES

The product has a typical nutritional value of approximately 170 kJ/100 g enzyme product.

- Carbohydrate 10 g/100 g
- Moisture 5 g/100 g

# CTL3 conc BG

## STORAGE CONDITION

**Recommended storage:** 0-10 °C (32-50 °F)

Packaging must be kept intact dry and away from sunlight. Please follow the recommendations and use the product before the best before date to avoid the need for a higher dosage.

**Best before:** You will find the best before date in the certificate of analysis or on the product label.

The product gives optimal performance when stored as recommended and used within 24 months of the production date.

Novozymes guarantees delivery at least 12 months prior to the best-before date.

## SAFETY AND HANDLING PRECAUTIONS

Enzymes are proteins. Inhalation of dust or aerosols may induce sensitization and may cause allergic reactions in sensitized individuals. Some enzymes may irritate the skin eyes and mucous membranes upon prolonged contact. See the MSDS or Safety Manual for further information regarding safe handling of the product and spills.

## COMPLIANCE

The product complies with the recommended purity specifications for food-grade enzymes given by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Food Chemical Codex (FCC).

Kosher and Halal certificates are available from the Customer Center or sales representative.

## CERTIFICATIONS

Novozymes is a signatory to United Nations Global Compact United Nations Convention on Biological Diversity and report on our sustainability performance through Global Reporting Initiative (GRI). See all our commitments under sustainability on [www.novozymes.com](http://www.novozymes.com).



## FOOD SAFETY

Novozymes has carried out a hazard analysis and prepared an HACCP plan describing the critical control points (CCPs). The HACCP plan is supported by a comprehensive prerequisite program implemented in Novozymes' GMP practices.

The product is produced according to Novozymes' HACCP plan GMP practices and additional requirements controlled by Novozymes' Quality Management System.

The product complies with FAO/WHO JECFA- and FCC-recommended purity requirements regarding mycotoxins. The product complies with EU legislation regarding pesticides.

The product is produced under FSSC 22000 certification.



## PACKAGING

The product is available in different types of packaging. Please contact the sales representative for more information.

Novozymes A/S  
Krogshøjvej 36  
2880 Bagsvaerd  
Denmark

Tel. +45 4446 0000  
Fax +45 4446 9999

For more information or for more office addresses visit [www.novozymes.com](http://www.novozymes.com)

Laws regulations and/or third party rights may prevent customers from importing using processing and/or reselling the products described herein in a given manner. Without separate written agreement between the customer and Novozymes to such effect this document does not constitute a representation or warranty of any kind and is subject to change without further notice.



TO WHOM IT MAY CONCERN

DIVISION OF  
FOOD QUALITY, TECHNOLOGY  
AND MARKETING PRACTICES



04.03.2010



**iZyme B**

The Danish Veterinary and Food Administration hereby certifies having accepted in March 2010 the enzyme product iZyme B from Novozymes A/S. The product, which is derived from a genetically modified strain of *Bacillus licheniformis* expressing the gene encoding a serine protease from *Nocardia prasina*, has been accepted to be used for protein hydrolysis at a level of 1500000 PROT per kg dry protein.

The evaluation of the safety of iZyme B has been made in accordance with the principles laid down in the Guidelines for the presentation of data on food enzymes, "cf. Reports of the Scientific Committee of Food, 27<sup>th</sup>, Series; EUR 14181, 1992.

Yours faithfully





To whom it may concern

DIVISION OF  
FOOD QUALITY, TECHNOLOGY  
AND MARKETING PRACTICES

31.03.2008

### **The evaluation process for safety of new enzymes**

The Danish Veterinary and Food Administration hereby certifies that the evaluation process of the safety of new enzymes is made in accordance with the principles laid down in the "Guidelines for the presentation of data on food enzymes" cf. Reports of the Scientific Committee of Food, 27<sup>th</sup> Series, EUR 14181, 1992.

In accordance with this information about the following is required and evaluated before acceptance of use of each new enzyme in food production in Denmark:

#### CONTEXT

#### ADMINISTRATIVE DATA

#### TECHNICAL DATA

- 1 Active components
  - 1.1 Primary enzyme activity
  - 1.2 The activity of the enzyme preparation
  - 1.3 Subsidiary enzymatic activities
- 2 Source materials
  - 2.1 Animal sources
  - 2.2 Plant sources
  - 2.3 Microbial sources
  - 2.4 Genetically modified organisms
- 3 Manufacturing Process
  - 3.1 Fermentation
  - 3.2 Purification
- 4 Carrier and other additives and ingredients
  - 4.1 Formulation - Ingredients and additives
  - 4.2 Immobilized enzyme preparation
  - 4.3 TOS and composition
- 5 Usage
  - 5.1 Technological function
  - 5.2 Types of foodstuffs
  - 5.3 Maximum dosage of the enzyme preparation
- 6 Stability and fate in the food
  - 6.1 Amount of enzyme in the final food preparation
  - 6.2 Main reaction products

- 6.3 Possible effects on nutrients
- GENERAL REQUIREMENTS AND SPECIFICATIONS
- 7 Hygiene
  - 7.1 Good Manufacturing Practice (GMP)
  - 7.2 Influence on total microbial count in final foodstuff
- 8 Purity specifications/absence of contaminants
  - 8.1 Heavy metals
  - 8.2 Microbiological contaminants
  - 8.3 Production organism
  - 8.4 Antibiotic activity
  - 8.5 Toxins

#### DOCUMENTATION FOR SAFETY IN USE

- 9 Basic toxicological requirements
  - 9.1 Enzymes derived from edible animals or plants
  - 9.2 Enzymes derived from micro-organisms
- 10 Exemptions from the basic toxicological requirements if relevant

#### EVALUATION OF SAFETY IN USE

- Estimate of human consumption
- Safety margin

#### LIST OF REFERENCES

The level of details required for the individual points are as given in the above mentioned guideline. For additional information regarding individual enzyme notifications, please contact the Danish Veterinary and Food Agency, Division of Food Quality, Technology and Marketing Practice.

Yours faithfully



Biochemist, PhD



## Appendix 3

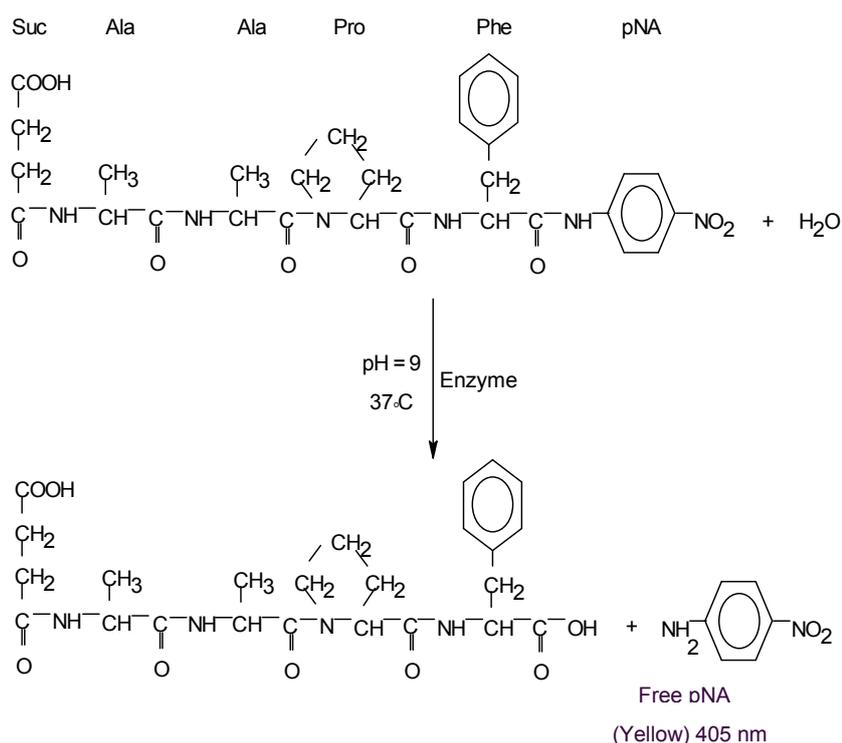
### Methods of analysis used to determine compliance with the specifications

1. Enzyme activity method, KMCU/g	2013-02314
2. KMCU activity units	2014-05866
3. Total aerobic viable count	EB-SM-3001.02
4. Total coliforms	EB-SM-3091.02
5. Escherichia coli (E. coli).	EB-SM-5036.02
6. Salmonella	EB-SM-3009.02
7. Antimicrobial activity	2014-05507
8. Production strain	EB-SM-3000.02
9. Heavy metals	UT015a

## Microbial Chymotrypsin-like activity on Konelab (KMCU)

### Principle

The serine endopeptidase hydrolyzes the substrate Suc-Ala-Ala-Pro-Phe-pNA. The release of yellow pNA results in an increase in absorbance at 405 nm, and this increase is proportional to the enzyme activity.



Parameter	Reaction conditions
Temperature	37°C
pH	9.0
Substrate conc.	Working solution: 0.56 mg/ml
Enzyme conc.	0.012–0.060 mKMCU/ml
Reaction time	250 s
Interval kinetic measuring time	Every 18 s 11 times, the first measurement after 60 s
Wavelength	405 nm

## Definition of unit

1 KMCU is defined relative to an enzyme standard.

## Equipment

Equipment	
Konelab 30 analyzer	Thermo Fisher Scientific
Diluter	E.g., Hamilton Microlab or pipettes
Analytical balance	E.g., Sartorius
pH meter	E.g., Radiometer, Metrohm
Magnetic stirrer plates	-

## Chemicals

Name	Chemical formula	Brand
Suc-Ala-Ala-Pro-Phe-pNA	$C_{30}H_{36}N_6O_9$	L-1400 Bachem
Dimethyl sulfoxide	$(CH_3)_2SO$	e.g. Sigma D-5879
Sodium chloride	NaCl	e.g. Merck 6404
Triton X-100	t-Oct- $C_6H_4-(OCH_2CH_2)_xOH$ , x=9-10	e.g. Sigma T9284
Citric acid monohydrate	$HOC(COOH)(CH_2COOH)_2, H_2O$	e.g. Merck 244
Trisodium citrate dihydrate	$HOC(COONa)(CH_2COONa)_2, 2(H_2O)$	e.g. Merck 6448
Tris base	$NH_2C(CH_2OH)_3$	Sigma 7-9 T1378

*EXAMPLE:* Check out the Material Safety Data Sheets (MSDSs) for the chemicals.

## Reagents

### Tris buffer:

*EXAMPLE:* Preparation of 1 L of Tris buffer 0.1M pH 9.00:

Step	Action
1	Pour approx. 500 ml of deionized water into a 1000-ml volumetric flask.
2	Weigh out 12.11 g of Tris and transfer it to the volumetric flask.
3	Weigh out 8.77 g of NaCl and transfer it to the volumetric flask.
4	Fill the flask to approx. 900 ml and stir.
5	While stirring add 10 ml 10% Triton X-100
6	<i>IMPORTANT:</i> The buffer temperature must be 23.0–25.0°C before pH measurement.
7	When the Triton X-100 has dissolved, adjust the pH to 9.00 ± 0.03 using 4M HCl.
8	Fill the volumetric flask with deionized water and stir.
9	Storability: 24 hr at room temperature. The diluent is adjusted to 23.0–25.0°C before use. If it is necessary to adjust the temperature, the solution can be stirred first before withdrawing a suitable amount for temperature adjustment.

### Suc-Ala-Ala-Pro-Phe-pNA stock solution:

Preparation of 1 ml of stock solution

Step	Action
1	Weigh out 50 mg of Suc-Ala-Ala-Pro-Phe-pNA substrate in a small beaker.
2	Add 1 ml of DMSO to the substrate. Add a magnet and cover the beaker with foil (as the substrate is sensitive to light). <i>NOTE:</i> Use nitrile gloves!
3	Put the beaker on a stirrer.
4	Storability: 1 day at room temperature.

### Suc-Ala-Ala-Pro-Phe-pNA working solution:

Preparation of 25 ml of working solution

Step	Action
1	Rinse a 25-ml volumetric flask with Tris buffer (see above) to prevent any contamination of the substrate.
2	Remove 350 µl of the substrate stock solution using a pipette and add it to the 25-ml volumetric flask. <i>NOTE:</i> Use nitrile gloves!
3	Fill the flask with Tris buffer (see above).
4	Mix the reagent by turning the flask upside down a couple of times. <i>NOTE:</i> Wrap the flask immediately in foil or use a dark volumetric flask as the reagent is sensitive to light.
5	Storability: 6 hr at room temperature when stored in the dark (with foil).

**Citrate buffer:**

*EXAMPLE:* Preparation of 1 L of 10 mM pH 3.40:

Step	Action
1	Fill a 1000-ml volumetric flask with approx. 500 ml of deionized water.
2	Weigh out 1.56 g of citric acid monohydrate and transfer it to the 1000-ml volumetric flask.
3	Weigh out 0.76 g of trisodium citrate dihydrate and transfer it to the volumetric flask.
4	Weigh out 8.77 g of sodium chloride and transfer it to the volumetric flask.
5	Fill the flask to approx. 900 ml with deionized water and stir.
6	While stirring add 10 ml 10% Triton X-100
7	If necessary, adjust the pH to 3.40 ± 0.03 using 2M NaOH once the Triton X-100 has dissolved.
8	Fill the flask with deionized water and stir.
9	Storability: 3 days at room temperature.

**Standards**

The standard is available on request.

**Preparation:**

Step	Action																																										
1	Stock solution: Weigh out an amount of enzyme standard corresponding to 0.750 KMCU units.																																										
2	Dissolve the standard in citrate buffer (10 mM, pH 3.40) in a 250-ml measuring flask.																																										
3	Stir for at least 15 min. Storability: 6 hr at room temperature.																																										
4	Working solutions: The standard curve is a seven-point curve with a factor of 5 between lowest and highest standard points. The recommended volume to make the standard curve series is 1500 µl.																																										
	<table border="1"> <thead> <tr> <th rowspan="2">Standard no.</th> <th rowspan="2">Dilution ratio</th> <th colspan="2">Example</th> <th rowspan="2">Concentration (mKMCU/ml)</th> </tr> <tr> <th>Stock solution (µl)</th> <th>Diluent (µl)</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>50</td> <td>30</td> <td>1470</td> <td>0.060</td> </tr> <tr> <td>2</td> <td>30</td> <td>50</td> <td>1450</td> <td>0.100</td> </tr> <tr> <td>3</td> <td>25</td> <td>60</td> <td>1440</td> <td>0.120</td> </tr> <tr> <td>4</td> <td>20</td> <td>75</td> <td>1425</td> <td>0.150</td> </tr> <tr> <td>5</td> <td>15</td> <td>100</td> <td>1400</td> <td>0.200</td> </tr> <tr> <td>6</td> <td>12</td> <td>125</td> <td>1375</td> <td>0.250</td> </tr> <tr> <td>7</td> <td>10</td> <td>150</td> <td>1350</td> <td>0.300</td> </tr> </tbody> </table>	Standard no.	Dilution ratio	Example		Concentration (mKMCU/ml)	Stock solution (µl)	Diluent (µl)	1	50	30	1470	0.060	2	30	50	1450	0.100	3	25	60	1440	0.120	4	20	75	1425	0.150	5	15	100	1400	0.200	6	12	125	1375	0.250	7	10	150	1350	0.300
Standard no.	Dilution ratio			Example			Concentration (mKMCU/ml)																																				
		Stock solution (µl)	Diluent (µl)																																								
1	50	30	1470	0.060																																							
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3	25	60	1440	0.120																																							
4	20	75	1425	0.150																																							
5	15	100	1400	0.200																																							
6	12	125	1375	0.250																																							
7	10	150	1350	0.300																																							
	Storability: 6 hr at room temperature.																																										

## QC Sample

The QC sample is available on request.

Prepare a QC sample with known enzyme content in the same way as for the samples below.

## Sample

Minimum dissolution factor: 100 ml/g.

Sample amount: 0.5–1.78 g.

### Preparation:

Step	Action
1	Weigh out the sample in a weighing boat and transfer it quantitatively to a volumetric flask using citrate buffer. The activity in the final dilution should be approx. 0.200 mKMCU/ml.
2	Fill the volumetric flask to the mark with citrate buffer and stir for minimum 15 min.
3	Storability: 6 hr at room temperature.

## Blank

No reagent blank.

## Procedure

Step	Action												
1	Prepare reagents, dilutions of standard, QC sample, and samples.												
2	Start up the Konelab 30.												
3	Place the reagents in the Konelab: <table border="1" data-bbox="453 1240 1251 1693"> <thead> <tr> <th>Reagent</th> <th>Konelab reagent name</th> <th>Reagent container volume*</th> <th>Syringe speed</th> </tr> </thead> <tbody> <tr> <td>Citrate buffer: 10 mM, pH 3.40 with 150 mM NaCl and 0.01% Triton X-100</td> <td>KMCU-BUF</td> <td>20 ml</td> <td>Normal</td> </tr> <tr> <td>Suc-Ala-Ala-Pro-Phe-pNA substrate, working solution</td> <td>KMCU-SUB</td> <td>10 ml</td> <td>Normal</td> </tr> </tbody> </table> <p><i>NOTE:</i> * If a smaller reagent container is used, no alarm will be given for shortage of reagent.</p>	Reagent	Konelab reagent name	Reagent container volume*	Syringe speed	Citrate buffer: 10 mM, pH 3.40 with 150 mM NaCl and 0.01% Triton X-100	KMCU-BUF	20 ml	Normal	Suc-Ala-Ala-Pro-Phe-pNA substrate, working solution	KMCU-SUB	10 ml	Normal
Reagent	Konelab reagent name	Reagent container volume*	Syringe speed										
Citrate buffer: 10 mM, pH 3.40 with 150 mM NaCl and 0.01% Triton X-100	KMCU-BUF	20 ml	Normal										
Suc-Ala-Ala-Pro-Phe-pNA substrate, working solution	KMCU-SUB	10 ml	Normal										
4	Place the standards, QC sample, and samples in the Konelab in the stated order. <i>NOTE:</i> 21 samples can be analyzed in a single analytical run.												
5	Start the Konelab.												

## Calculations

Step	Action
1	The activity of the enzyme samples is determined relative to the standard curve.
2	On the basis of the results of Abs/min for the seven standards, a standard curve is drawn with the activities of the standards in mKMCU/ml as the x-values and the Abs/min of the standards as the y-values. The standard curve is described by a Logit-Log4 fit.
3	<p>The enzyme activity of the diluted samples is read from the standard curve. The activity of a sample in KMCU/g is calculated using the formula:</p> $\text{Activity KMCU/g} = \frac{S \times V \times F}{W \times 1000}$ <p>S = Reading from the standard curve in mKMCU/ml            V = Volume of the measuring flask in ml            F = Dilution factor for second dilution            W = Weight of sample in g            1000 = Conversion factor from mKMCU to KMCU</p>
4	<p><i>EXAMPLE:</i> 0.5181 g of sample is dissolved in a 250-ml measuring flask and further diluted 300 times using a diluter. From the standard curve, an activity of 0.216 mKMCU/ml is calculated from the signal in Abs/min.</p> $\text{Activity} = \frac{0.216 \text{ mKMCU/ml} \times 250 \text{ ml} \times 300}{0.5181 \text{ g} \times 1000} = 31.27 \text{ KMCU/g}$

## Approval of analytical run

### Standard curve:

Parameter	Requirement
Quality of fit (lower $r^2$ limit)	$R^2 \geq 0.9945$
Curve appearance	The standard curve is a Logit-Log4 standard curve.

### QC sample:

The measured activity of the QC sample must be the declared value +/- 3 standard deviations.

### Samples:

The analytical result is an average of two weighings on two different standard curves.

## Statement of analysis results

The results are stated with three significant digits.

## Configurations

Conc.  
Time

## Handling of enzymes and chemicals

Enzymes and enzyme solutions should be handled in a fume hood or in closed containers.

Avoid inappropriate handling of enzymes and enzyme solutions, which may result in aerosol/dust generation.

Avoid inhalation of dust aerosols and contact with skin and eyes.

Handling of chemicals and disposal of waste must be performed according to valid procedures.

## Validity

Valid from February 2013

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Novozymes A/S  
Krogshøjvej 36  
2880 Bagsvaerd  
Denmark

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Fax +45 4446 9999

For more information  
and addresses of  
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[info@novozymes.com](mailto:info@novozymes.com)

Novozymes is the world leader in bioinnovation. Together with customers across a broad array of industries we create tomorrow's industrial biosolutions, improving our customers' business and the use of our planet's resources. Read more at [www.novozymes.com](http://www.novozymes.com).

Novozymes file: 2014-05866

## **KMCU activity units**

Relationship between activity units KMCU and activity units PROT

PROT was the activity unit designation used at the time the toxicological testing of the serine protease from *Nocardopsis prasina* expressed in *Bacillus licheniformis* was conducted. This is reflected in the toxicological study reports where the activity unit is designated as PROT. Since then, the activity unit designation has been changed into KMCU which is short for **Kilo Microbial Chymotrypsin Units**. KMCU corresponds directly to PROT, but is a 1000 factor lower due to the Kilo unit. Therefore:

1 KMCU = 1/1000 PROT

Both activities are measured by the same analytical method given in Appendix 3.1, "Analytical method for Microbial Chymotrypsin like activity on Konelab (KMCU)"; it is simply the unit designation that has been changed.

## Enumeration of Total Viable Count

**Scope** All Novozymes Enzyme Business QC laboratories involved in analysis of samples from Novozymes production and GLP studies.

**Principle** **Total Viable Count (TVC)** is defined as the number of organisms which form colonies on a non-selective agar medium (Tryptic Soy Agar, TSA) after aerobic incubation for 3 days at 30-35°C. TSA is a rich non-selective agar medium. The method outlined below conforms to the principles of (Ref. 1) with the following exceptions:

- The test only covers the enumeration of microorganisms capable of growing on TSA (Total aerobic Microbial Count).
- The dilution water has an addition of 4% Tween 80.
- EP describes the use of duplicates. This method uses single tests.
- The agar plates are incubated for 3 days, not for 3-5 days.
- Growth promotion test of TSA is performed according to in-house procedures and not according to the description in EP.

Routine samples are analysed by the spiral plater (100 µl) or spread plate technique (100 µl or 1 ml) as described below:

Sample type	Requested test (LIMS code)	Technique	Volume spread	Lowest Dilution	No. of plates	Plate size	Detection limit
Enzyme samples and fluid hyaluronic acid	TVC or TVC(ML)	Spiral plating or spread plating	100 µl	10 <sup>-1</sup>	1 plate	9 cm	100 CFU / g or ml
	TVC(100)	Spread plating	1 ml	10 <sup>-1</sup>	4 plates	14 cm	10 CFU / g or ml
CIP-samples	CIP_TVC	Spiral plating or spread plating	100 µl	Undiluted	1 plate	9 cm	10 CFU / ml
		Petrifilm	1 ml		N/A	N/A	1 CFU / ml
FeF samples	FEF_TVC	Spread plating	1 ml	10 <sup>-1</sup>	4 plates	14 cm	10 CFU / g or ml

Depending on sample type, level of contamination and the detection limit needed for the specific sample, alternative procedures may be used.

**IMPORTANT:** Petrifilm must only be used to analyze CIP samples if pH of the CIP water is within range 6.6-8.5 (Ref. 4 and 5).

*Continued on next page*

## Enumeration of Total Viable Count, *Continued*

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**Definition of units**

The result is stated as:

- Total Viable Count (TVC) / g or ml

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**Samples**

All sample types.

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**Detection limit**

The detection limit of this method is dependent on the sample volume and the dilution in use (See "Principle").

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**Equipment**

- Balance ( $\pm 0.1$  g)
  - Magnetic stirrer
  - Petri dishes (9 cm or 14 cm)
  - Suitable sterile pipettes for transfer of 100  $\mu$ l or 1 ml (4x0.25 ml)
  - Spiral plater (for the spiral plate technique)
  - Sterile Drigalski spreaders (for the spread plate technique)
  - Incubator (30-35°C)
  - Stereo microscope or microscope
  - Plastic spreader (*Petrifilm test*)
- 

**Media and reagents**

- Tween 80 buffer 4%, 90 ml (if necessary with a magnet) prepared acc. to [EB-ME-0052](#)
  - EP buffer, 90 ml buffered sodium chloride-peptone solution pH 7.0, prepared acc. to [EB-ME-0067](#)
  - TSA plates (9 or 14 cm) prepared acc. to [EB-ME-0041](#)
  - 3M™ Petrifilm™ Aerobic Count Plates (*Petrifilm test*)
- 

**Safety**

It is the responsibility of the laboratory leader, that all personnel are aware of the correct handling of enzymes and reagents.

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*Continued on next page*

## Enumeration of Total Viable Count, *Continued*

### Sample preparation

Enzyme samples and other solid samples are prepared as follows:

Sample type	Action
<b>Enzyme samples</b> <b>FeF samples</b> <b>Other solid samples</b>	Transfer 10 g of solid sample or 10 ml of liquid sample to 90 ml Tween 80 buffer 4%.  <i>NOTE:</i> Immediately homogenize the sample by stirring or by shaking. Solid samples are homogenized on a magnetic stirrer for app. 20 minutes though min. 1 hour for Sweetzyme (batch code IA).
<b>Non-enzyme fluid samples (e.g. CIP samples)</b>	Non-enzyme fluid samples are analyzed undiluted. If needed, 10-fold dilutions may be prepared with Tween 80 buffer 4%.
<b>Fluid hyaluronic acid (HA)</b>	Transfer 10 ml of liquid sample to 90 ml EP buffer. <i>IMPORTANT:</i> Homogenize on a magnetic stirrer for min. 1 hour. It is recommended to shake the sample after approx. 30 min.

*TIP:* All enzyme products must be analyzed from at least a  $10^{-1}$  dilution due to possible inhibition of microorganisms in undiluted enzyme. If an enzyme product is known to contain growth inhibiting components (e.g. rodalone or proxel) consider analyzing further dilutions prepared with Tween 80 buffer 4% (e.g.  $10^{-2}$  and  $10^{-3}$  dilutions). In this case be aware that the quantification limit is lower than the spec. limit of the sample.

*IMPORTANT:* Valid for US laboratories: TVC analysis must also be performed using a  $10^{-2}$  dilution if the spec. limit of the sample is  $> 30.000$  and/or for samples from Recovery 1 and 2.

*Continued on next page*

## Enumeration of Total Viable Count, *Continued*

### Plating

Plating must be done within 15 minutes from end of homogenisation. If this is not possible, the sample can be stored at 2-8°C for up to 4 hours.

Test	Action
<b>TVC</b>	Transfer 100 µl from the 10 <sup>-1</sup> dilution onto the surface of a TSA plate (9 cm). Repeat this for any of the necessary dilutions. <i>Or</i> Perform a spiral plating of 100 µl from the 10 <sup>-1</sup> dilution in accordance with the directions for the specific spiral plater.
<b>TVC(100)</b> <i>or</i> <b>TVC_FeF</b>	Transfer 1 ml from the 10 <sup>-1</sup> dilution onto the surface of 4 TSA plates (14 cm) with app. 0.25 ml onto each plate. Repeat this for any of the necessary dilutions.
<b>TVC_CIP using TSA plates</b>	Transfer 100 µl from the undiluted sample onto the surface of a TSA plate (9 cm). Repeat this for any of the necessary dilutions. <i>Or</i> Perform a spiral plating of 100 µl from the undiluted sample in accordance with the directions for the specific spiral plater.

Leave the plates on the table with lid on until the sample has been soaked into the agar.

Test	Action
<b>TVC_CIP using Petrifilm</b>	<ol style="list-style-type: none"> <li>1. Transfer 1 ml from the undiluted sample to the center of the film.</li> <li>2. Place plastic spreader, recessed side down, on center of sample and press down, gently and firmly to distribute inoculum.</li> <li>3. Wait at least one minute for gel to form</li> </ol>

### Incubation

Incubate the TSA agar plates at 30-35°C for 3 days.

Incubate the Petrifilm with clear side up at 35-39°C for 2 days.

*Continued on next page*

## Enumeration of Total Viable Count, *Continued*

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### Reading

#### **TSA agar plates – Spread plate technique:**

Count the number of colonies on the plates.

Size of agar plate	Count colonies on plates with
9 cm	1–300 colonies per plate
14 cm	1–750 colonies per plate

#### **TSA agar plates – Spiral plate technique:**

The number of typical colonies on each plate is counted and the result is calculated in accordance with the directions for the specific spiral plater. Danish sites may refer to (Ref. 2).

*IMPORTANT:* Small colonies, e.g. lactobacillus, may erroneously be misread as product crystallizations. If in doubt use stereo microscope for macroscopic observation and/or prepare a slide culture of a colony for light microscopy.

#### **Petrifilm**

Count the number of colonies on the film. Interval of reading is 1-250 colonies (Ref. 5).

*IMPORTANT:* Discoloration from enzyme residues may occur. In case this is observed the result must be considered invalid.

*TIP:* Refer to (Ref. 5) to get familiarized with reading Petrifilms.

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*Continued on next page*

## Enumeration of Total Viable Count, *Continued*

### Calculation

#### General principles:

The calculation is based on the number of colonies ( $C_x$ ) on the plate, and the sample volume analysed ( $V_x$ ).

The result is stated with two significant figures (e.g.  $2.2 \times 10^1$ ).

When Using results from	Then the result is	Where
One dilution	$\frac{C_x}{V_x}$	$C_x$ = no. of colonies $V_x$ = volume analysed
2 or more dilutions	$\frac{C_1 + C_2}{V_1 + V_2}$	$C_1$ = no. of colonies in lowest dilution $C_2$ = no. of colonies in next dilution $V_1$ = volume analyzed in lowest dilution $V_2$ = volume analyzed in next dilution

**IMPORTANT:** When using more than one dilution, the numbers from each dilution are compared (the likelihood of product inhibitions, contamination of the sample, analytical errors etc. is considered). In general, the highest dilution is used. If the result is stated on the basis of other dilutions, the reason must be given in the raw data.

When the sample volume is 0.1 ml then  $V_x$  and  $C_x$  are:

Dilution	Undiluted	$10^{-1}$	$10^{-2}$
$V_x$	0.1 ml	0.01 ml	0.001 ml
$C_x$	No. of colonies on the plate	No. of colonies on the plate	No. of colonies on the plate

**EXAMPLE:** Examples of calculating spread plate of 0.1 ml sample:

$C_x$	$V_x$ (g or ml)	Dilution	Result
0	0.01	$10^{-1}$	$\frac{<1}{0.01} = < 100$ / g or ml
123	0.1	$10^0$	$\frac{123}{0.1} = 1.2 \times 10^3$ / g or ml
334	0.01	$10^{-1}$	$\frac{>300}{0.01} = > 3.0 \times 10^4$ / g or ml
253 24	0.01 0.001	$10^{-1}$ $10^{-2}$	$\frac{253+24}{0.01+0.001} = 2.5 \times 10^4$ / g or ml

*Continued on next page*

## Enumeration of Total Viable Count, *Continued*

### Calculation (*continued*)

When the sample volume is 1 ml (four 14 cm agar plates with 0.25 ml on each plate) then  $V_x$  and  $C_x$  are:

Dilution	Undiluted	$10^{-1}$	$10^{-2}$
$V_x$	1 ml	0.1 ml	0.01 ml
$C_x$	sum of colonies on the 4 plates	sum of colonies on the 4 plates	sum of colonies on the 4 plates

*EXAMPLE:* Examples of calculating spread plate of 1 ml sample:

$C_x$	$V_x$ (g or ml)	Dilution	Result
0	0.1	$10^{-1}$	$\frac{<1}{0.1} = < 10 / \text{g or ml}$
123	1	$10^0$	$\frac{123}{1} = 1.2 \times 10^2 / \text{g or ml}$
426	0.1	$10^{-1}$	$\frac{426}{0.1} = 4.3 \times 10^3 / \text{g or ml}$
3134	0.1	$10^{-1}$	$\frac{>3000}{0.1} = > 3.0 \times 10^4 / \text{g or ml}$
853 84	0.1 0.01	$10^{-1}$ $10^{-2}$	$\frac{853+84}{0.1+0.01} = 8.5 \times 10^3 / \text{g or ml}$

*NOTE:* Calculation at Danish laboratories may follow:

- Spread plating of 1 ml: [PSL-MSP-0069](#)
- Spread plating of 100  $\mu\text{l}$ : [PSL-MSP-0082](#)
- Spiral plating of 100  $\mu\text{l}$ : [PSL-MSP-0075](#)

### Accuracy and precision

CV% (surface plating) = 25%

CV% (spiral plating) = 29%

*REFERENCE:* LUNA no. [2003-34435](#)

### Filing

All documentation should be filed in accordance with the local filing SOP.

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## Enumeration of Total Viable Count, *Continued*

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**Contingencies** All deviations from this SOP should be discussed with the Method Responsible Scientist and should be documented.

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- References**
1. European Pharmacopoeia, Chapter 2.6.12. Microbiological examination of non-sterile products (Total viable aerobic count).
  2. [PSL-MSP-0075](#): Beregning ved anvendelse af spiralplater (In Danish).
  3. [PSL-TE-3001](#): Spiralplater (In Danish).
  4. LUNA No. [2010-19643-01](#): Validation of pH Range Adjustment for Water Samples Using Petrifilm.
  5. [3M Petrifilm Interpretation Guide](#)
  6. [3M Petrifilm™ Aerobic Count Plates](#)
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**Revision** Added the LIMS Method TVC(ML) in the section "Principle". Flow chart added. Other minor editorial changes.

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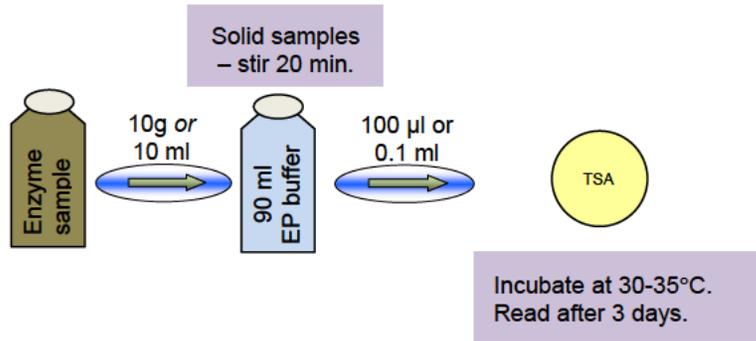
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## Enumeration of Total Viable Count, *Continued*

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### Appendix – Flow Chart

Flow chart of method for enzymes samples (LIMS method “TVC” or “TVC(ML)”). Click  to read section.



## Enumeration of coliform bacteria using Violet Red Bile agar

**IMPORTANT** This method is used for the analysis of **Sweetzyme** (batch code IA) and **liquid products** (with the exception of Biofeed Plus, batch code CN).

**Scope** All Novozymes QC laboratories involved in analysis of samples from Novozymes production and GLP studies.

**Principle** **Coliform bacteria** are broadly defined as Gram-negative, oxidase-negative, non-sporogenous rods, which grow in aerobic or facultative anaerobic conditions. More specifically, coliforms are capable of fermenting lactose (due to production of galactosidase) in the presence of bile at 37°C. Coliforms are not a taxonomically defined group of bacteria and consequently there is not a common agreement of which microorganisms truly belong to the coliform bacteria. However, as defined in (Ref. 3), Novozymes define coliform bacteria as organisms belonging to the genera *Escherchia*, *Citrobacter*, *Enterobacter*, *Klebsiella*, *Serratia* and *Hafnia*. The presence of coliform bacteria, especially *E. coli*, can be used as an indicator of the bacteriological hygiene of an enzyme product.

The Violet Red Bile agar (VRB) is a selective and indicative agar:

Principle	Description
Selective principle	Crystal violet and bile salts inhibit growth primarily of the Gram-positive accompanying flora. This favors growth of the fast growing Gram-negative enterobacteria.
Indicative principle	Degradation of lactose to acid is indicated by the pH indicator neutral red, which changes its color to red and in some cases also by precipitation of bile acids. Coliform bacteria degrade lactose.

Routine testing is performed in the following way:

Sample type	Requested test (LIMS code)	Technique	Volume spread	Lowest dilution	No. of plates	Plate size	Detection limit
Enzyme samples	COLIFORM	Pour plate with cover layer	2½ ml	10 <sup>-1</sup>	1 plate	14 cm	4 CFU / g or ml
CIP and water samples	CIP_ COLIFORM	Pour plate with cover layer	1 ml	Undiluted	1 plate	9 cm	1 CFU / g or ml

Depending on sample type, level of contamination and the detection limit needed for the specific sample, alternative procedures may be used.

*Continued on next page*

## Enumeration of coliform bacteria using Violet Red Bile agar, *Continued*

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### Principle (*continued*)

The method outlined below conforms to ISO 4832 with the following deviations:

- ISO 4832 and ISO 6887-1 describe the use of a Peptone-salt-solution or Buffered-peptone-water as diluent. This Novozymes method uses Tween80 buffer 4%.
  - ISO 4832 describes the use of duplicates. This Novozymes method uses single tests.
- 

### Definition of units

The result is stated as:

- Coliform bacteria / g or ml
- 

### Samples

This method is used for the analysis of **Sweetzyme** (batch code IA) and **liquid products** (with the exception of Biofeed Plus, batch code CN). Biofeed Plus is analyzed according to [EB-SM-3005](#).

*NOTE:* In addition, the method can be used for analysis of certain solid samples (e.g. cryst. conc. T).

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### Detection limit

The detection limit of this method is dependent on the sample volume and the dilution in use (See the section "Principle").

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### Equipment

Balance ( $\pm 0.1$  g)  
Magnetic stirrer  
Petri dishes (9 cm or 14 cm)  
Suitable sterile pipette for transfer of 1 ml or 10 ml (4 x 2.5 ml)  
Incubator (34-38°C)

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## Enumeration of coliform bacteria using Violet Red Bile agar, *Continued*

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### Media and reagents

- Tween80 buffer 4%, 90 ml (If necessary, with a magnet) prepared acc. to [EB-ME-0052](#).
- Violet Red Bile agar (VRB) prepared acc. to [EB-ME-0051](#) or [NZNAQC-2.05.10a](#) (US Labs).

*NOTE:* If the VRB agar is freshly prepared in the laboratory, suspend the media with 200 ml ion exchanged water and leave to bulk for 15 min. Suspension of VRB agar should be executed in a clean bench to avoid inhalation of the powder. Ensure that media is thoroughly dissolved before melting procedure by regular shaking of the media. In addition, stir the agar immediately before cooling in water bath and again before pouring in Petri dishes.

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### Safety

*NOTE:* It is the responsibility of the laboratory leader, that all personnel are aware of the correct handling of enzymes and reagents.

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### Sample preparation

Enzyme samples are prepared as follows:

Step	Action
1	Transfer 10 g of solid sample or 10 ml of liquid sample to 90 ml Tween80 buffer 4%.
2	Immediately homogenize the sample by stirring or by shaking. Solid samples are homogenized on a magnetic stirrer for app. 20 min.

*IMPORTANT:* All enzyme products must be analyzed from a  $10^{-1}$  dilution due to possible inhibition of microorganisms in undiluted enzyme.

Non-enzyme liquid samples (e.g. CIP-samples) are analyzed undiluted.

*TIP:* Further 10-fold dilutions of any sample type can be prepared with Tween80 buffer 4%.

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## Enumeration of coliform bacteria using Violet Red Bile agar, *Continued*

### Plating

Plating is performed using the pour plate technique:

Sample type	Description
Enzymes	<ol style="list-style-type: none"> <li>1. Transfer 2½ ml from the 10<sup>-1</sup> dilution to an empty Petri dish (14 cm).</li> <li>2. Pour app. 40-45 ml VRB agar (47 ± 2°C) in the Petri dish (= bottom layer) and mix carefully. Leave this to solidify.</li> <li>3. Pour app. 10-15 ml VRB agar (47 ± 2°C) onto the bottom layer (= covering layer). Leave this to solidify.</li> </ol>
CIP and water samples	<ol style="list-style-type: none"> <li>1. Transfer 1 ml from the undiluted sample to an empty Petri dish (9 cm).</li> <li>2. Pour app. 20-25 ml VRB agar (47 ± 2°C) in the Petri dish (= bottom layer) and mix carefully. Leave this to solidify.</li> <li>3. Pour app. 5-10 ml VRB agar (47 ± 2°C) onto the bottom layer (=covering layer). Leave this to solidify</li> </ol>

### Incubation

Incubate the plates at 34-38°C for 22- 26 hours at aerobic conditions.

### Reading

Count the number of typical colonies on the agar plate after 22 – 26 hours incubation, calculate the result, and register the reading.

Size of agar plate	Count colonies on plates with	Typical colonies
9 cm	1-150 per plate	Purplish red with a diameter of ≥ 0.5 mm and sometimes surrounded by a reddish zone of precipitated bile.
14 cm	1-375 per plate	

In case of doubt, the colonies should be examined in microscope, as e.g. enterococci might grow in VRB. Coliform bacteria will appear as small rods.

**NOTE:** Pink pin-point colonies may be enterococci, possibly *Klebsiella* cf. Merck application brochure. *Aeromonas* also grow on VRB agar and produce red colonies but without a precipitation zone of bile salts. *Aeromonas* can only be distinguished from coliform bacteria by testing for presence of oxidase. However, verification is not performed in this method.

*Continued on next page*

## Enumeration of coliform bacteria using Violet Red Bile agar, *Continued*

### Calculation

#### General principles:

The calculation is based on the number of colonies ( $C_x$ ) on the plate, and the sample volume analyzed ( $V_x$ ).

The result is stated with two significant figures (e.g.  $2.2 \times 10^1$ ).

When Using results from	Then the result is	Where
One dilution	$\frac{C_x}{V_x}$	$C_x$ = no. of colonies $V_x$ = volume analyzed
2 or more dilutions	$\frac{C_1 + C_2}{V_1 + V_2}$	$C_1$ = no. of colonies in lowest dilution $C_2$ = no. of colonies in next dilution $V_1$ = volume analyzed in lowest dilution $V_2$ = volume analyzed in next dilution

**IMPORTANT:** When using more than one dilution, the numbers from each dilution are compared (the likelihood of product inhibitions, contamination of the sample, analytical errors etc. is considered). In general, the highest dilution is used. If the result is stated on the basis of other dilutions, the reason must be given in the raw data.

**When the sample volume is 1 and 2½ ml, respectively, then  $V_x$  is:**

Dilution	Volume	Undiluted	$10^{-1}$	$10^{-2}$
$V_x$	1 ml	1 ml	0.1 ml	0.01 ml
$V_x$	2½ ml	N/A	0.25 ml	0.025 ml

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## Enumeration of coliform bacteria using Violet Red Bile agar, *Continued*

### Calculation (continued)

*EXAMPLE:* Calculation of 1 ml sample on a 9 cm agar plate:

<b>C<sub>x</sub></b>	<b>V<sub>x</sub></b> (g or ml)	<b>Dilution</b>	<b>Result</b>
0	1	10 <sup>0</sup>	$\frac{0}{1} = < 1 / \text{g or ml (LIMS = < 10)}$
18	1	10 <sup>0</sup>	$\frac{18}{1} = 18 / \text{g or ml}$

*EXAMPLE:* Calculation of 2½ ml sample on a 14 cm agar plate:

<b>C<sub>x</sub></b>	<b>V<sub>x</sub></b> (g or ml)	<b>Dilution</b>	<b>Result</b>
0	0.25	10 <sup>-1</sup>	$\frac{0}{0.25} = < 4 / \text{g or ml (LIMS = < 10)}$
1	0.25	10 <sup>-1</sup>	$\frac{1}{0.25} = 4 / \text{g or ml}$
3	0.25	10 <sup>-1</sup>	$\frac{3}{0.25} = 12 / \text{g or ml}$
412	0.25	10 <sup>-1</sup>	$\frac{375}{0.25} = > 1.5 \times 10^3 / \text{g or ml}$
53 8	0.25 0.025	10 <sup>-1</sup> 10 <sup>-2</sup>	$\frac{53+8}{0.25+0.025} = 2.2 \times 10^2 / \text{g or ml}$

*IMPORTANT:* When the result entered in LIMS is a 'less than' value lower than < 10 / g or ml, LIMS will automatically change this value to "< 10".

### Accuracy and precision

CV% = 29%

*REFERENCE:* [LUNA No. 2003-34435](#)

### Filing

All documentation should be filed in accordance with the local filing SOP.

### Contingencies

All deviations from this SOP should be discussed with the Method Responsible Scientist and should be documented.

*Continued on next page*

## Enumeration of coliform bacteria using Violet Red Bile agar, *Continued*

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### References

1. ISO 4832 2<sup>nd</sup> Ed. (1991) Microbiology – General Guidelines for the enumeration of coliforms – colony count technique.
  2. ISO 6887-1 1<sup>st</sup> Ed. (1999) Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspensions and decimal dilutions for microbiological examination – Part 1: General rules for the preparation of the initial suspension and decimal dilutions.
  3. [LUNA no. 2009-26425-01](#): Definition of enterobacteria and coliform bacteria at Novozymes.
- 

### Revision

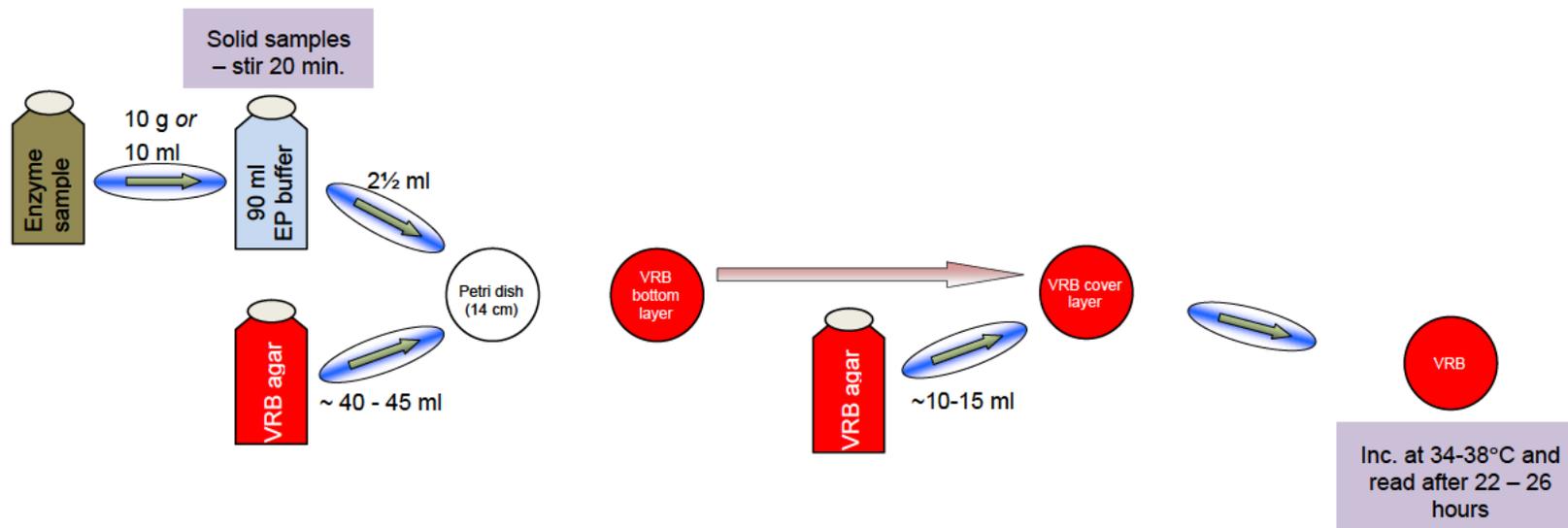
Elaborated on preparation of VRB agar and reference to preparation of VRB agar in US laboratories in the section “Media and reagents”. Changed amount of agar used in the section “Plating” to harmonize with EB-SM-3004 (VRBD agar). Changed incubation time from 1 day to 22 – 26 hours to minimize risk of false negative results – see LUNA No. [2013-04384-01](#). Added note about growth of non-coliforms and use of microscopy to distinguish enterococci from coliform bacteria in the section “Reading”. Minor editorial changes.

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## Enumeration of coliform bacteria using Violet Red Bile agar, *Continued*

Appendix – Flow chart of method analyzing an enzyme sample. Ctrl + Click  to read section.  
Flow chart



## Detection of *E. coli* in 25 g

**Scope** All Novozymes QC laboratories involved in analysis of samples from Novozymes production and GLP studies.

**Overview**

Section	Section
<a href="#">Principle</a>	<a href="#">Manual IMS (O157)</a>
<a href="#">Standards</a>	<a href="#">Automated IMS (O157)</a>
<a href="#">Equipment</a>	<a href="#">Detection on CT-SMAC/Chrom agar</a>
<a href="#">Media and Reagents</a>	<a href="#">Verification Latex (O157)</a>
<a href="#">Safety</a>	<a href="#">Interpretation of results</a>
<a href="#">Transfer of sample to BPW</a>	<a href="#">Action on Results</a>
<a href="#">Enrichment</a>	<a href="#">Revision</a>
<a href="#">Detection on TBX</a>	<a href="#">Flow Chart of method</a>

**Principle**

***Escherichia coli* (*E. coli*)** is a Gram-negative, indole positive, facultative anaerobic rod. It is considered a faecal indicator.

Detection of *E.coli* in 25 g is carried out as a qualitative analysis using non-selective enrichment in Buffered Peptone Water (BPW) followed by isolation of  $\beta$ -D-glucuronidase positive *E.coli* (blue-green or dark-blue to violet colonies) on a selective indicative agar medium (TBX agar).  $\beta$ -Glucuronidase-negative *E. coli* strains (3-4 %) form colourless colonies on TBX agar, e.g. *E. coli* O157. The detection of *E.coli* O157 is performed as ImmunoMagnetic Separation (IMS) using Dynabeads®antiO157 and plating onto two selective indicative agar media (CT-SMAC agar and ChromAgar O157). Suspect *E.coli* O157 colonies are verified using *E.coli* O157 Latex test.

Typical colonies from TBX agar and/or *E.coli* O157 Latex positive isolates from CT-SMAC agar and/or ChromAgar O157 are reported as *E. coli* Detected in 25 g

Typical colonies are further verified for Enterovirulent *E. coli* (EEC).

**Requested test (LIMS code): E.COLI(25g)**

**IMPORTANT:** *E. coli* (25g) analysis is usually requested together with Enterovirulent *E. coli* (EEC), Lims code: EV *E.coli*. If *E. coli* (25g) is Not Detected (ND) both methods are reported as ND in LIMS. Samples from “Re-processed batches” (*E. coli* detected first time) may come without the EV.*E.coli* analysis (already performed).

*Continued on next page*

## Detection of *E. coli* in 25 g, *Continued*

### Principle (continued)

The media used has the following characteristic:

Media...	Characteristic...
BPW broth	Non-selective broth.
TBX agar	<p><u><i>E.coli</i> colonies are coloured blue-green.</u></p> <p>Growth of accompanying Gram-positive flora is largely inhibited by the use of bile salts.</p> <p>The presence of the enzyme <math>\beta</math>-D-glucuronidase differentiates most <i>E.coli</i> spp. from other coliforms. <i>E.coli</i> absorbs the chromogenic substrate 5-bromo-4-chloro-3-indolyl-<math>\beta</math>-D-glucuronide (X-<math>\beta</math>-D-glucuronide). The enzyme <math>\beta</math>-glucuronidase splits the bond between the chromophore 5-bromo-4-chloro-3-indolyle- and the <math>\beta</math>-D-glucuronide. <i>E.coli</i> colonies are coloured blue-green.</p> <p><i>NOTE:</i> For the recovery of sub lethally injured <i>E. coli</i>, plates are incubated at 34 - 38°C and not 44°C as recommended by Merck (inhibits growth of accompanying Gram-positive flora).</p>
CT- SMAC agar (MacConkey Sorbitol agar)	<p><u>Sorbitol negative bacterial (including O157:H7) colonies are transparent and almost colourless with a pale yellowish-brown appearance.</u></p> <p>Polypeptone favours the growth of Escherichia coli O157:H7. Most other bacteria are inhibited by the association of bile salts, crystal violet, cefixime and potassium tellurite.</p> <p>Sorbitol positive bacteria give rise to red colonies, due to the change of the colour of the pH indicator (neutral red).</p>
ChromAgar O157 and CT-Chrom Agar O157	<p><u>A typical <i>E.coli</i> O157 will grow as a pale lavender-lilac colour (mauve) colony.</u></p> <p>Most other bacteria are inhibited or grow as blue or colourless colonies.</p>

### Definition of units

The result is stated as:

- DET (*E.coli* Detected in 25 g) or
- ND (*E.coli* Not Detected in 25 g) together with EV. *E. coli* ND (if requested)

### Samples

All Novozymes sample types except hygiene samples.

*Continued on next page*

## Detection of *E. coli* in 25 g, *Continued*

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**Standards** A positive reference strain can be used, e.g. *E. coli*, ATCC 11229.  
If a reference strain of *E. coli* O157 is included, it must be *E. coli* O157 without the genes coding for Vero Toxins, e.g. ATCC 43888

---

**Detection limit** Theoretical detection limit: 1 *E. coli* in 25 g

---

**Equipment** Balance ( $\pm 0.1$  g)  
Magnetic stirrer  
Incubator (34-38°C)  
Incubator or Waterbath for pre-heating BPW (40-42°C)  
Sterile inoculation loops (1 and 10  $\mu$ l)  
Sterile swabs  
Vortex mixer  
Pipettes and sterile tips  
For ImmunoMagnetic Separation (either mIMS or aIMS):

- For manual ImmunoMagnetic Separation (mIMS):
  - MPC-S Rack and magnet (Invitrogen Cat. No. 120.20) + Eppendorf tubes 1.5 ml (Eppendorf Cat. No. 0030 10.086) + MX-3 Mixer (Dynal Cat. No. 159.09), mixer is optional.
- For automatic ImmunoMagnetic Separation (aIMS):
  - BeadRetriever (Invitrogen Cat. No. 159-50) + Tubes & tips (Invitrogen Cat. No. 150-51)

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Continued on next page

## Detection of *E. coli* in 25 g, *Continued*

### Media and reagents

- Buffered Peptone Water (BPW) (450 ml) prepared acc. to [EB-ME-0009](#) (Danish sites: [MSA-SUB-FS-0427](#))
- Chromocult®TBX agar plates (9 cm) prepared acc. to [EB-ME-0012](#) (Danish sites: [MSA-SUB-FS-04275](#))
- Cefixime-Tellurite-Sorbitol MacConkey agar -CT-SMAC (9 cm) prepared acc. to [EB-ME-0015](#) (Danish sites: [MSA-SUB-FS-0453](#))
- ChromAgar O157 agar plates (9 cm) or CT-ChromAgar O157 (5 or 9 cm) prepared acc. to [EB-ME-0014](#) (Danish sites: [MSA-SUB-FS-0454](#))
- Tryptone Soya agar plates (TSA) prepared acc. to [EB-ME-0041](#) (Danish sites: [MSA-SUB-FS-0260](#))
- Dynabeads®anti O157, Dynal Cat. 710.04
- Washing buffer (PBS-Tween 20 buffer), Sigma No. P-3563
- E.coli O157 Latex test kit (for verification), Oxoid No. DR620

**IMPORTANT:** Preparation in the local laboratory shall be done according to the current valid WW Media direction.

### Safety

- It is the responsibility of the laboratory leader that all personnel are aware of the correct handling of enzymes and reagents.
- E. coli O157 Latex test (Oxoid DR0620) is Harmful if swallowed (CLP H302) due to 0.1% Sodium azide.

### Transfer of sample to BPW

• If the sample is ...	Then transfer 25 g sample to ...
Novamyl SM30 conc. BG (AB.....) Neutrase 1.5 Unstd M (PW.....) Ultraflo Unstd MG (CN .....) Clear Lens Pro 2.5 MG (P.....) Viscoflow MG (KR .....) Flavourzyme 500 MG (HP .....) Ceremix Plus MG (WD .....)	900 ml BPW  (Use two 450 ml BPW bottles and transfer 12.5 g to each bottle)
... any other sample	450 ml BPW

**NOTE:** For liquid samples 25 ml may be used.

*Continued on next page*

## Detection of *E. coli* in 25 g, *Continued*

### Enrichment

The non-selective enrichment may be performed in the following ways:

- Incubate sample in BPW at 34-38°C for 16-24 hours

OR

- Incubate sample in pre-heated BPW at 34-38°C for 16-20 hours.
  - Pre-heating of BPW may take place in water bath (min. 1 hour) or incubator (min. 4 hours) at 40-42°C.

**IMPORTANT:** Pre-heated BPW is introduced to make it possible to incubate sample for *Salmonella* and *E. coli* in the same bottle when *Salmonella* is analyzed using PCR.

**IMPORTANT:** For GLP samples (dept. 402) only pre-heated BPW is used.

### Detection of $\beta$ -D-glucuronidase positive *E. coli*

Detection of  $\beta$ -D-glucuronidase positive *E. coli* is performed in the following way:

- Streak the enriched sample onto the surface of a TBX agar plate using a sterile 10  $\mu$ l inoculation loop. If 2 BPW bottles streak on 1 agar plate from each bottle.
- Incubate the plate at 34-38°C for 1 day (min. 18 hours).
- Examine the plate for growth of typical *E. coli* colonies:

Organism	Growth on Chromocult®TBX agar
<i>E. coli</i>	Blue-green or dark-blue to violet colonies colonies (Salmon-GAL and X-glucuronide reaction)
<i>Coliforms</i> (not <i>E. coli</i> )	Salmon to red colonies (Salmon-GAL reaction but no X-glucuronide reaction )
<i>Other Gram-negatives</i> (not <i>E. coli</i> )	Colourless colonies, except for some organisms which possess $\beta$ -D-glucuronidase activity. These colonies appear light-blue to turquoise.

*Continued on next page*

## Detection of *E. coli* in 25 g, *Continued*

### Detection of *E.coli* O157

**ImmunoMagnetic Separation (IMS) is performed either as manual IMS (= mIMS) or as automated IMS (= aIMS):**

#### Manual IMS (= mIMS):

Step	Action
1	Place an Eppendorf tube per sample in the rack without the magnet inserted. Gently vortex the Dynabeads®anti O157, and add 20 µl Dynabeads®anti O157 to each tube. Use a lid opener for opening the lids of the Eppendorf tubes.
2	Gently add 1 ml of the pre-enriched sample to the Eppendorf tube. Use a new pipette / pipette tip for each sample. Close the lid. <i>NOTE:</i> If sample is divided in 2 BPW bottles take 0.5 ml from each bottle.
3	Incubate the tubes for app. 10 minutes at room temperature. The rack is gently rotated without the magnet on a MX-3 Mixer (Dynal) or by hand.
4	Insert the magnet in the rack. Tilt the rack frequently for app. 3 minutes to ensure a complete collection of beads. With correct capture a distinct circular to oval brownish pellet is formed at the tube site halfway between the top and bottom of the tube.
5	Open the tubes gently by use of the lid opener. Place a Pasteur pipette at the water surface opposite to the pellet. Gently pipette up the supernatant and the liquid in the cap of the tube. Slow down pipetting when the surface of the liquid passes the pellet in order to make sure that no beads leave the tube through the pipette. If beads leave the sample, return the supernatant to the tube and repeat step 4. Use a new pipette / pipette tip for each sample.
6	Carefully add 1 ml of washing buffer to each sample. Do not touch the tube with the pipette / pipette tip since this can cross-contaminate the samples as well as the buffer. Close the lids and remove the magnet from the rack. Wash the bead complex by rotating the rack 3 times. Repeat step 4-6 twice, but the last time the pellet is only re-suspended in 100 µl washing buffer.

***Continued on next page***

## Detection of *E. coli* in 25 g, *Continued*

### Detection of *E. coli* O157 (*continued*)

#### Automatic ImmunoMagnetic Separation (AIMS):

Step	Action
1	<p>Load one sample tube for each sample into a sample rack.</p>  <p><i>NOTE:</i> Each sample tube consists of 5 tubes called tube 1-5 (tube 1 is to the left (= slip end), and tube 5 is to the right).</p>
2	Gently vortex the Dynabeads®anti O157 until the pellet in the bottom of the tube disappears, and aseptically add 10 µl properly mixed Dynabeads®anti O157 into sample tubes 1 and 2.
3	Aseptically add 0.5 ml of wash buffer to sample tubes 1 and 2. Aseptically add 1 ml of wash buffer to sample tubes 3 and 4. Aseptically add 100 µl of wash buffer to sample tube 5.
4	Add 0.5 ml of the enriched test sample to sample tubes 1 and 2, be careful not to contaminate other tubes. If sample is divided in 2 BPW bottles take 0.5 ml from each bottle.
5	Repeat step 4 for the remaining samples.
6	Aseptically insert the sterile protective sample tip combs into the instrument.
7	Insert the rack with filled tubes into the instrument to lock it in place.
8	Check that everything is properly aligned. Close the instrument door
9	Select the EPEC/VTEC program sequence by scrolling with the arrow key, and press the Start button.

*NOTE:* Check that all beads have been transferred to tube no. 5, some sample types can interfere with the IMS. If all beads haven't been transferred to tube 5, repeat *Step 9*. If the problem remains, repeat aIMS with 4 tubes instead of 1 (125µl sample to tube 1 and 2 in 4 tubes). When finished, transfer material from all tube no 5 into 1 tube and continue.

## Detection of *E. coli* in 25 g, *Continued*

### Detection of *E.coli* O157 (*continued*)

#### Streaking onto selective indicative agar plates:

Each IMS product (from mIMS or from aIMS) is tested for the presence of *E.coli* O157 using selective indicative agar plates:

Step	Action
1	Gently vortex the pellet (IMS-product).
2	Transfer 50 µl IMS-product onto the surface of a CT-SMAC agar plate, and another 50 µl IMS-product to the surface of a ChromAgar O157 plate (or a CT-ChromAgar O157 plate) in the following way: Spread the bead-bacteria complex over one half of the plate with a sterile cotton swab. This ensures the break-up of the bead-bacteria complexes. Dilute further by streaking with a loop.
3	<ul style="list-style-type: none"> <li>Incubate the plates at 34-38°C for 1 day (min. 18 hours).</li> </ul>

#### Reading:

Agar	Description
CT-SMAC agar	<p>On CT-SMAC agar, typical <i>E.coli</i> O157 colonies are transparent and almost colourless with a pale yellowish-brown appearance and a diameter of approximately 1 mm.</p> <p>Sorbitol positive organisms form bright red (pink) colonies.</p> <p>In some cases suspect colonies are so few that they can only be recognized in the bacterial lawn in the primary streaking zone. In this case, subculture suspect colony material onto a new CT-SMAC agar plate.</p> <p>If the growth is too weak after 1 day, the plates can be re-incubated for up to 24 hours. In this case representative sorbitol negative colonies (transparent) shall be verified by use of the <i>E.coli</i> O157 Latex kit from Oxoid (see below).</p>
ChromAgar O157 and CT-Chrom Agar O157	A typical <i>E.coli</i> O157 will grow as a pale lavender-lilac colour (mauve) colony, whereas most other microorganisms are either inhibited or grow as blue or colourless colonies.

*Continued on next page*

## Detection of *E. coli* in 25 g, *Continued*

### Detection of *E. coli* O157 (*continued*)

#### Verification of *E. coli* O157: Latex test

Suspect colonies on CT-SMAC agar and ChromAgar O157 (or CT-ChromAgar O157) are verified as suspect *E. coli* O157 using O157 Latex test kit from Oxoid. The test will demonstrate by slide agglutination *E. coli* strains possessing the O157 serogroup antigen

Step	Action
1	Bring latex reagents to room temperature and mix well
2	Control of <i>E. coli</i> O157 latex test: Positiv control: Mix 1 drop Test latex and 1 drop Positive control suspension on test card. Rock card for maximum 1 minute, agglutination should occur within 1 minute Negative control: Mix 1 drop Test latex and 1 drop Negative control suspension on test card. Rock card for maximum 1 minute, agglutination should not occur within 1 minute
3	Test of suspect colony: 1. 1 drop of Test latex and 1 drop of 0.85 % Saline solution onto the reaction card close to the circle border, do not mix! 2. Dissolve 1 colony thoroughly in the Saline solution and mix with Test latex solution 3. Rock the reaction card for maximum 1 minute, agglutination should occur within 1 minute 4. If agglutination occurs (do not use a magnifying glass) the colony is latex positive (i.e. suspect <i>E. coli</i> O157).
4	<b>IMPORTANT:</b> Test for auto-agglutination (only if step 3 is positive): 1. 1 drop of Control latex mixture and 1 drop of 0.85 % Saline solution onto the reaction card close to the circle border, do not mix! 2. Dissolve 1 colony thoroughly in the Saline solution and mix with Control latex solution 3. Rock the reaction card for maximum 1 minute 4. If agglutination occurs the colony is auto-agglutinating and the reaction in step 3 is a false positive (i.e. colony is latex negative)

**IMPORTANT:** Agglutination should occur within 1 minute and should be visible without use of magnifying glass

**NOTE:** Dept. 402 continues directly to EV. *E. coli* PCR Verification ([PSL-SM-3097](#)) and dept. 60895 (Franklinton) may choose ([EB-SM-3097](#))

*Continued on next page*

## Detection of *E. coli* in 25 g, *Continued*

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### Interpretation of results

#### *E. coli* Detected (DET) in 25 g

- Presence of typical colonies on TBX agar
- Presence of O157 Latex positive colonies from CT-SMAC agar and ChromAgar O157 (or CT-ChromAgar O157), i.e. suspect *E. coli* O157.

#### *E. coli* Not Detected (ND) in 25 g

- Absence of typical colonies on TBX agar
  - Absence of O157 Latex positive colonies from CT-SMAC agar and ChromAgar O157 (or CT-ChromAgar O157).
- 

### Action on results

#### *E. coli* Detected (DET) in 25 g

- Report *E. coli* DET in 25 g

If EV. *E. coli* is requested on sample:

- Streak suspect colonies onto TSA agar and incubate 34-38°C for 18-24 h.  
*Note:* Dept. 575 (DK) may send selective plate with suspect colonies, when all plates for a given sample are completed to Dept. 402.
- Send TSA plate for verification to:  
Novozymes A/S Mikrobiologisk control Dept. 402  
Krogshoejvej 36, building 1KS.18  
DK-2880 Bagsvaerd
- Send an E-mail to "mkelab" and "JAah" (Responsible Scientist) stating:  
E. coli Detected (subject line) and LIMS no. and/or ID no. of isolate
- Dept. 402 will enter verification result for EV. *E. coli* in LIMS directly for all sites if not otherwise agreed.

**IMPORTANT:** Department 60895 Franklinton may perform the EV. *E. coli* verification for Franklinton/Blair samples.

#### *E. coli* Not Detected (ND) in 25 g

- Report *E. coli* ND in 25 g
- Report EV. *E. coli* ND in 25 g (if requested in LIMS)

**IMPORTANT:** If *E. coli* DET in GLP samples contact Responsible Scientist

**IMPORTANT:** GLP samples are reported in GLP study documentation.

**IMPORTANT:** EV. *E. coli* Detected in Finished Goods must be reported to Product Quality Management (PQM)

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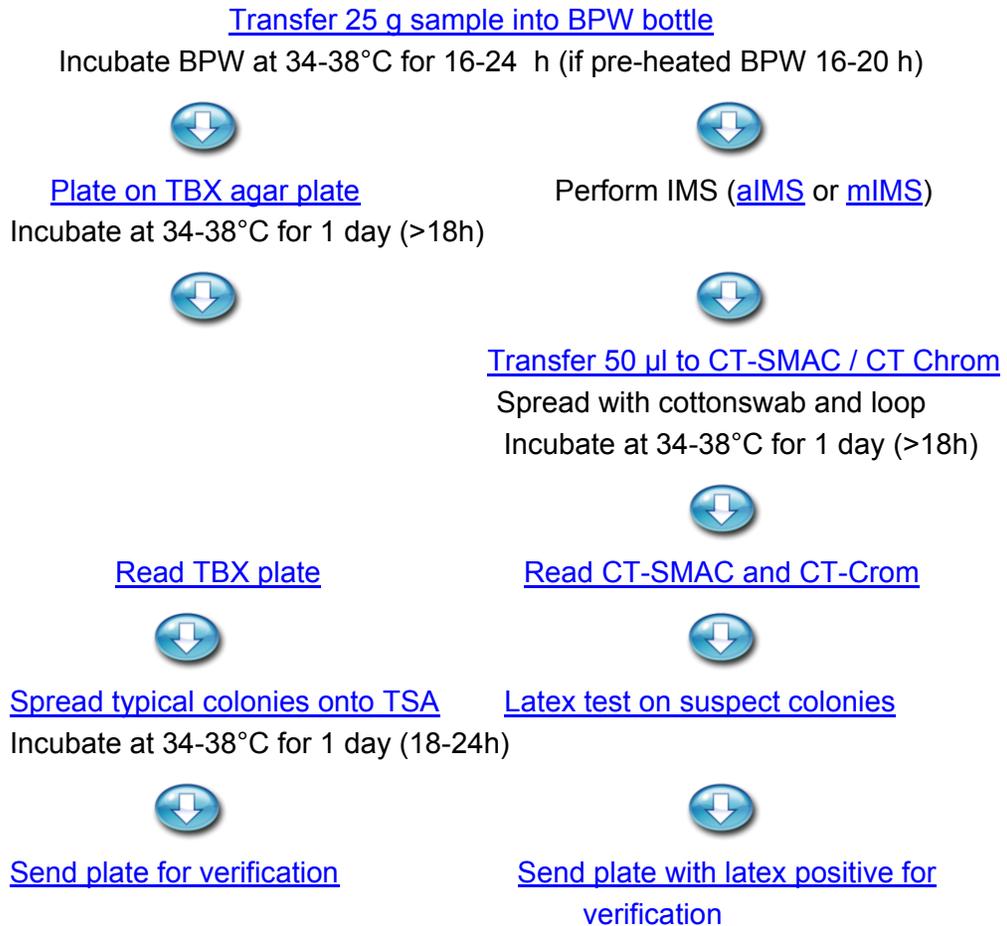
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## Detection of *E. coli* in 25 g, *Continued*

### Flow chart

Flow chart of method, Click [Link](#) to read section.



Result	LIMS	Action
No typical colonies on TBX or CT-SMAC/CT-Chrom	<i>E. coli</i> (25g) ND EV. <i>E. coli</i> ND	Analysis is completed
No typical colonies on TBX and no latex positive from CT-SMAC/CT-Chrom		
Typical colonies on TBX or latex positive colonies from CT-SMAC/CT-Chrom	<i>E. coli</i> (25g) DET	Send plate for EV. <i>E. coli</i> verification (if requested)

## Detection of *Salmonella* spp. by cultivation

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**Scope** All Novozymes QC laboratories involved in analysis of samples from Novozymes production and GLP studies.

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Section	Section
1. <a href="#">Principle</a>	10. <a href="#">Selective enrichment in RVs broth</a>
2. <a href="#">Definition of units</a>	11. <a href="#">Selective enrichment mIMS</a>
3. <a href="#">Sample types</a>	12. <a href="#">Selective enrichment aIMS</a>
4. <a href="#">Standards</a>	13. <a href="#">Detection</a>
5. <a href="#">Detection limit</a>	14. <a href="#">Reading plates</a>
6. <a href="#">Safety</a>	15. <a href="#">Verification</a>
7. <a href="#">Equipment and materials</a>	16. <a href="#">API identification</a>
8. <a href="#">Media and reagents</a>	17. <a href="#">Interpretation of results</a>
9. <a href="#">Non-selective enrichment</a>	18. <a href="#">Flow Chart</a>

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**Principle** Detection of *Salmonella* spp. is carried out as a qualitative test. Requested test (LIMS code): SALMONELLA.

The test is based on a non-selective enrichment in Buffered Peptone Water for 18-24 hours followed by selective enrichment in RVs broth or immunomagnetic separation of *Salmonella* followed by a cultivation method with the *Salmonella* specific XLD and Rambach agar plates. Suspect *Salmonella* colonies are then verified using Oxidase test and API 20E or API Rapid 20E.

All methods are in-house methods evaluated and validated at Novozymes.

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**Definition of units** The result is stated as:

- DET (*Salmonella* detected in 25 gram)
- ND (*Salmonella* not detected in 25 gram)

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**Sample types** All Novozymes samples from production and GLP studies.

**IMPORTANT:** Hygiene samples are analyzed according to [EB-SM-5001](#).

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## Detection of *Salmonella* spp. by cultivation, *Continued*

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**Standards** A positive reference strain can be included in the test, e.g. *Salmonella adabraka*, *Salmonella havana* or *Salmonella senftenberg*.

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**Detection limit** Theoretical detection limit: 1 *Salmonella* spp. in 25 g.

---

**Safety** It is the responsibility of the laboratory leader that all personnel are aware of the correct handling of enzymes and reagents.

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**Equipment and materials**

**General equipment**

- Balance
- Incubator for BPW and agar plates (34-38°C)
- Incubator or water bath for BPW or RVs (40.0-42.0°C)
- Vortex mixer
- Automatic pipettes and sterile tips (10-100 µl, 100-1000 µl, and 1 ml)
- Sterile inoculation loops (1 and 10 µl)

---

**Media and reagents**

**Enrichment broths**

- Buffered Peptone Water (BPW) (450 ml) prepared acc. to [EB-ME-0009](#)
- Rappaport Vassiliadis soya peptone broth (RVs broth) (approx. 10 ml) (Oxoid CM0866)

**Agar plates and reagents**

- XLD agar plates prepared acc. to [EB-ME-0069](#) or (Oxoid Cat. No. PO5057A)
- Rambach agar plates prepared acc. to [EB-ME-0033](#)
- BS (Brilliance *Salmonella*) agar plates (Oxoid Cat. No. PO5098A)
- Tryptic Soy agar plates (TSA) prepared acc. to [EB-ME-0041](#)
- Reagent for oxidase test, e.g. Bactident oxidase (Merck Cat. No. 1.13300.0001)
- API Rapid 20E (BioMérieux Cat. No. 20 701) or API 20 E (BioMérieux Cat. No. 20 100) + relevant API reagents

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## Detection of *Salmonella* spp. by cultivation, *Continued*

### Non-selective enrichment

The non-selective enrichment is performed in the following way:

Step	Description						
1	<p>Transfer 25 g or 25 ml sample to 450 ml BPW equilibrated to room temperature.</p> <p><i>IMPORTANT:</i> If any of these products transfer 25 g to 900 ml or 2x12.5g to 2x450 ml BPW bottles.</p> <ol style="list-style-type: none"> <li>1. Bio-Feed Alpha CT (AD.....)</li> <li>2. Bio-Feed Wheat L*H*NbulkL DK (CF.....)</li> <li>3. Ceremix Plus MG (WD.....)</li> <li>4. Clear Lens Pro 2.5 MG (P.....)</li> <li>5. Flavourzyme 500 MG (HP.....)</li> <li>6. Neutrased 1.5 MG (PW.....)</li> <li>7. Novamyl SM30 conc. BG (AB.....)</li> <li>8. Ronozyme HiPhos GT (HK.....)</li> <li>9. Viscoflow MG (KR.....)</li> </ol>						
2	<p>Cultivation method:</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 50%;">If...</th> <th style="width: 50%;">Then...</th> </tr> </thead> <tbody> <tr> <td>Selective enrichment in RVs broth is chosen</td> <td>Incubate BPW at 34-38°C for 16 - 24 hours</td> </tr> <tr> <td>Selective enrichment using IMS is chosen</td> <td>Incubate BPW at 34-38°C for 22 - 26 hours</td> </tr> </tbody> </table>	If...	Then...	Selective enrichment in RVs broth is chosen	Incubate BPW at 34-38°C for 16 - 24 hours	Selective enrichment using IMS is chosen	Incubate BPW at 34-38°C for 22 - 26 hours
If...	Then...						
Selective enrichment in RVs broth is chosen	Incubate BPW at 34-38°C for 16 - 24 hours						
Selective enrichment using IMS is chosen	Incubate BPW at 34-38°C for 22 - 26 hours						

### Selective enrichment in RVs broth

The selective enrichment in RVs is performed in the following way:

Step	Description
1	<p>Transfer 100 µl or 0.1 ml from BPW to 10 ml RVs tubes equilibrated to minimum room temperature.</p> <p><i>NOTE:</i> If 2 BPW bottles are used for one sample transfer 50 µl from each bottle to one RVs broth</p>
2	<p>Incubate the RVs broth at 40.0-42.0°C for 22 - 26 hours.</p> <p><i>NOTE:</i> If water bath is used to incubate RVs there is no need to equilibrate the temperature of the RVs broth.</p>

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## Detection of *Salmonella* spp. by cultivation, *Continued*

### Selective enrichment mIMS

The selective enrichment using mIMS is performed the following way:

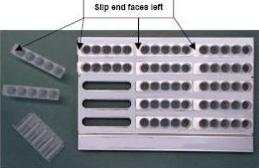
Step	Description
1	Place an Eppendorf tube per sample in the rack without the magnet inserted. Gently vortex the Dynabeads®anti-Salmonella and add 20 µl Dynabeads®anti-Salmonella to each tube.
2	Gently add 1 ml of the pre-enriched sample to the Eppendorf tube. Use a new pipette / pipette tip for each sample. Close the lid. If sample is enriched in 2 BPW bottles transfer 500 µl from each bottle.
3	Incubate the tubes for app. 10 min. at room temperature. The rack is gently rotated without the magnet on a MX-3 Mixer (or similar) or by hand.
4	Insert the magnet in the rack. Tilt the rack frequently for app. 3 min. to ensure a complete collection of beads. With correct capture a distinct circular to oval brownish pellet is formed at the tube site halfway between the top and bottom of the tube.
5	Open the tubes gently by use of the lid opener. Place a Pasteur pipette at the water surface opposite to the pellet. Gently pipette up the supernatant and the liquid in the cap of the tube. Slow down pipetting when the surface of the liquid passes the pellet in order to make sure that no beads leave the tube through the pipette. If beads leave the sample, return the supernatant to the tube and repeat step 4. Use a new pipette / pipette tip for each sample.
6	Carefully add 1 ml of washing buffer to each sample. Do not touch the tube with the pipette / pipette tip since this can cross-contaminate the samples as well as the buffer. Close the lids and remove the magnet from the rack. Wash the bead complex by rotating the rack 3 times. Repeat step 4-6 twice, but the last time the pellet is only re-suspended in 100 µl wash buffer.

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## Detection of *Salmonella* spp. by cultivation, *Continued*

### Selective enrichment aIMS

The selective enrichment using aIMS is performed the following way:

Step	Description
1	<p>Load one sample tube for each sample into a sample rack.</p>  <p>Each sample tube consists of 5 tubes called tube 1-5 (tube 1 is to the left (= slip end), and tube 5 is to the right).</p>
2	<p>Gently vortex the Dynabeads®anti-Salmonella until the pellet in the bottom of the tube disappears, and aseptically add 10 µl properly mixed Dynabeads®anti-Salmonella into sample tubes 1 and 2.</p>
3	<p>Aseptically add 500 µl of wash buffer to sample tubes 1 and 2. Aseptically add 1000 µl of wash buffer to sample tubes 3 and 4. Aseptically add 100 µl of wash buffer to sample tube 5.</p>
4	<p>For each sample remove the labelled sample tube strip from the sample rack, and place it in a second sample rack. Add 500 µl of the enriched test sample to sample tubes 1 and 2, and return the inoculated tube strip to the first sample rack.</p> <p>If sample is enriched in 2 BPW bottles transfer 500 µl from each bottle.</p> <p><b>CAUTION:</b> Be careful not to cross contaminate, if possible place racks well separated.</p>
5	<p>Repeat step 4 for the remaining samples.</p>
6	<p>Aseptically insert the sterile protective sample tip combs into the instrument.</p>
7	<p>Insert the rack with filled tubes into the instrument to lock it in place.</p>
8	<p>Check that everything is properly aligned. Close the instrument door.</p>
9	<p>Select the Salmonella program sequence by scrolling with the arrow key, and press the Start button.</p>

**NOTE:** Check that all magnets have been transferred to tube 5 as this may not occur always with some difficult sample types. If all magnets have not been transferred to tube 5, then repeat step 9. If the problem remains then repeat step 1-9 using four sample tubes instead of one. Add only 125 µl enriched test sample to tubes 1 and 2 in each sample tube. Transfer all material from tubes no. 5 into one of the no. 5 tubes (approx. 400 µl in total).

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## Detection of *Salmonella* spp. by cultivation, *Continued*

### Detection

RVs broth is tested for the presence of *Salmonella* spp. using two different selective indicative agar plates (XLD agar and Rambach or BS agar):

Step	Description							
<b>1</b>	<table border="1"> <thead> <tr> <th style="background-color: #0070C0; color: white;">If...</th> <th style="background-color: #0070C0; color: white;">Then...</th> </tr> </thead> <tbody> <tr> <td>RVs</td> <td>Mix (vortex) RVs broth.</td> </tr> <tr> <td>IMS</td> <td>Mix (vortex) the pellet IMS-product.</td> </tr> </tbody> </table>		If...	Then...	RVs	Mix (vortex) RVs broth.	IMS	Mix (vortex) the pellet IMS-product.
If...	Then...							
RVs	Mix (vortex) RVs broth.							
IMS	Mix (vortex) the pellet IMS-product.							
<b>2</b>	<table border="1"> <thead> <tr> <th style="background-color: #0070C0; color: white;">If...</th> <th style="background-color: #0070C0; color: white;">Then...</th> </tr> </thead> <tbody> <tr> <td>RVs</td> <td>Streak 10 µl RVs broth using a 10 µl inoculation loop onto the surface of a XLD agar plate, and streak another 10 µl RVs broth to the surface of a Rambach or BS agar plate. Same inoculation loop may be used.</td> </tr> <tr> <td>IMS</td> <td>Streak 50 µl IMS-product onto the surface of a XLD agar plate, and streak another 50 µl IMS-product to the surface of a Rambach agar plate.  Spread the bead-bacteria complex over one half of the plate with a sterile cotton swab. This ensures the break-up of the bead-bacteria complexes. Dilute further by streaking with an inoculation loop (1 µl).  <i>NOTE:</i> If IMS has been performed using four sample tubes then streak 200 µl IMS-product on each of the XLD and Rambach agar plates.</td> </tr> </tbody> </table>		If...	Then...	RVs	Streak 10 µl RVs broth using a 10 µl inoculation loop onto the surface of a XLD agar plate, and streak another 10 µl RVs broth to the surface of a Rambach or BS agar plate. Same inoculation loop may be used.	IMS	Streak 50 µl IMS-product onto the surface of a XLD agar plate, and streak another 50 µl IMS-product to the surface of a Rambach agar plate.  Spread the bead-bacteria complex over one half of the plate with a sterile cotton swab. This ensures the break-up of the bead-bacteria complexes. Dilute further by streaking with an inoculation loop (1 µl).  <i>NOTE:</i> If IMS has been performed using four sample tubes then streak 200 µl IMS-product on each of the XLD and Rambach agar plates.
If...	Then...							
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IMS	Streak 50 µl IMS-product onto the surface of a XLD agar plate, and streak another 50 µl IMS-product to the surface of a Rambach agar plate.  Spread the bead-bacteria complex over one half of the plate with a sterile cotton swab. This ensures the break-up of the bead-bacteria complexes. Dilute further by streaking with an inoculation loop (1 µl).  <i>NOTE:</i> If IMS has been performed using four sample tubes then streak 200 µl IMS-product on each of the XLD and Rambach agar plates.							
<b>3</b>	Incubate the plates at 34-38°C for 1 day (minimum 18 hours).							

*Continued on next page*

## Detection of *Salmonella* spp. by cultivation, *Continued*

### Reading plates

Agar	Description	
<p><b>Rambach agar</b></p>		<p>Selective principle: Na-desoxycholate inhibit gram-positive flora.</p> <p>Indicative principle: Contains pH indicator, propylene glycol and chromogene.</p> <p><a href="#">Rambach agar</a></p> <p>Salmonella</p> <ul style="list-style-type: none"> <li>Red colonies – produce acid from propylene glycol.</li> </ul> <p><i>NOTE:</i> <i>S. arizona</i> form brownish, green-purple or blue-purple colonies.</p> <p>Coliform</p> <ul style="list-style-type: none"> <li>Blue-violet/Blue-green colonies – presence of β-D-galactosidase.</li> </ul> <p>Other enterobacteriaceae and gram-negative bacteria (<i>Proteus</i>, <i>Pseudomonas</i>, <i>Shigella</i>, <i>S. typhi</i> and <i>S. paratyphi A</i>)</p> <ul style="list-style-type: none"> <li>Colourless-yellow colonies.</li> </ul>
<p><b>XLD</b></p>		<ul style="list-style-type: none"> <li>Selective principle: Na-desoxycholate inhibits gram-positive flora and coliforms.</li> <li>Indicative principle: Contains lysine and H<sub>2</sub>S indicators.</li> </ul> <p><a href="#">XLD agar</a></p> <p>Salmonella</p> <ul style="list-style-type: none"> <li>Black colonies surrounded by a transparent, glass-like edge (black colour due to H<sub>2</sub>S-production).</li> </ul> <p><i>NOTE:</i> Some strains of <i>S. havana</i> form gray-brownish colonies (“fish-eye”) as they are H<sub>2</sub>S-negative.</p> <p><i>NOTE:</i> Be aware of very small black pin-point Salmonella colonies.</p> <p><i>Shigella</i>, <i>Providencia</i>, H<sub>2</sub>S-negative <i>Salmonella</i> (Ref. 3) and some <i>Proteus</i> and <i>Pseudomonas</i></p> <ul style="list-style-type: none"> <li>Red colonies.</li> </ul> <p>Other colonies may be white, greyish black or transparent.</p>

*NOTE:* Rambach and XLD agar may be stored at cool for up to 48 hours before reading cf. (Ref. 2).

*Continued on next page*

## Detection of *Salmonella* spp. by cultivation, *Continued*

Reading plates ( <i>continued</i> )	Agar	Description
	<b>BS agar</b>	<div data-bbox="542 369 889 724" data-label="Image"> </div> <p data-bbox="932 363 1429 531">                     Selective principle: Inhibigen inhibit <i>E. coli</i>. Other compounds, e.g. novobiocin and cefsulodin, suppress other bacteria (e.g. <i>Proteus</i> and <i>Pseudomonas</i>) and yeasts.                 </p> <p data-bbox="932 541 1429 674">                     Indicative principle: Contains two different chromogenes targeting caprylate esterase and <math>\beta</math>-D-glucosidase activities.                 </p> <p data-bbox="932 724 1429 758"> <a href="#">Brilliance Salmonella agar</a> </p> <p data-bbox="542 779 1429 884"> <i>Salmonella</i> <ul style="list-style-type: none"> <li>• Purple to pink colonies – presence of caprylate esterase.</li> </ul> <i>NOTE:</i> <i>S. dublin</i> form colorless to light grey-brown colonies.                 </p> <p data-bbox="542 934 1429 1003"> <i>Klebsiella</i>, <i>Enterobacter</i>, <i>Serratia</i> <ul style="list-style-type: none"> <li>• Blue colonies – presence of <math>\beta</math>-D-glucosidase.</li> </ul> </p> <p data-bbox="542 1045 1429 1106"> <i>Citrobacter</i>, other bacteria and yeasts                     <ul style="list-style-type: none"> <li>• White or colourless colonies</li> </ul> </p>

### Verification

**IMPORTANT:** Verification using **API Rapid 20E** tests must always be performed using colonies subcultivated on TSA agar plates incubated at 34-38°C for 1 day.

**IMPORTANT:** Verification using **API 20E** is traditionally executed with pure colonies cultivated on a non selective agar plate such as TSA agar. However, according to (Ref. 1 and 2) it is possible to perform verification using API 20E directly from the selective XLD, Rambach and BS agar plates.

*Continued on next page*

## Detection of *Salmonella* spp. by cultivation, *Continued*

### Verification (continued)

Hence, verification using API 20E is performed as follows:

Step		Description							
Day 1	If...	Then...							
A	A suspect colony is present as a single, pure, colony on XLD, Rambach or BS	<ol style="list-style-type: none"> <li>1. Perform <a href="#">API 20E test</a> using a single, pure colony, from XLD, Rambach or BS agar plate.</li> <li>2. Subcultivate from the same colony on a TSA agar plate. Incubate at 34-38°C for 1 day.</li> </ol>							
B	A suspect colony is present but <u>not</u> as a single, pure, colony on XLD, Rambach or BS	<ol style="list-style-type: none"> <li>1. Streak suspect colony onto new XLD, Rambach and/or BS agar plate and a TSA agar plate. Incubate all plates at 34-38°C for 1 day.</li> <li>2. <table border="1"> <thead> <tr> <th>If...</th> <th>Then...</th> </tr> </thead> <tbody> <tr> <td>Pure colony on TSA<sup>1</sup></td> <td>Proceed with step Day 2B.</td> </tr> <tr> <td>Pure colony on XLD, Rambach or XLD</td> <td>Proceed with step Day 1A.</td> </tr> </tbody> </table> </li> </ol> <p><b>CAUTION:</b> <sup>1</sup> It may be difficult to identify a <i>Salmonella</i> colony on TSA agar if more than one colony type is present on the agar plate.</p>		If...	Then...	Pure colony on TSA <sup>1</sup>	Proceed with step Day 2B.	Pure colony on XLD, Rambach or XLD	Proceed with step Day 1A.
If...	Then...								
Pure colony on TSA <sup>1</sup>	Proceed with step Day 2B.								
Pure colony on XLD, Rambach or XLD	Proceed with step Day 1A.								

*Continued on next page*

## Detection of *Salmonella* spp. by cultivation, *Continued*

**Verification  
(continued)**

		Description	
Day 2	If...	Then...	
A	A suspect colony is present as a single, pure, colony on XLD, Rambach or BS	1. Perform <a href="#">oxidase test</a> on colony material from TSA agar plate.	
		2.	
		Oxidase test is positive	Result is ND
		Oxidase test is negative	Read API 20E test and determine <a href="#">API ID</a> .
B	A suspect colony is present but <u>not</u> as a single, pure, colony on XLD, Rambach or BS	1. Perform <a href="#">oxidase test</a> on colony material from TSA agar plate.	
		2.	
		Oxidase test is positive	Result is ND
		Oxidase test is negative	Perform <a href="#">API 20 E test</a> using a single, pure colony, from TSA agar plate.
Day 3	If...	Then...	
B	A suspect colony is present but <u>not</u> as a single, pure, colony on XLD, Rambach or BS	Read API 20E and determine <a href="#">API ID</a> .	

**IMPORTANT:** Verification must always be performed using freshly grown cultures, i.e. verification may not be performed from agar plates stored at cool. If selective agar plates have been stored at cool before reading, fresh cultures must be prepared by subcultivation of suspect colonies on a TSA agar plate incubated at 34-38°C for 1 day.

*Continued on next page*

## Detection of *Salmonella* spp. by cultivation, *Continued*

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### Verification (*continued*)

#### Verification Kits

Local procedure SOP for Oxidase and API may be used, e.g. [Oxidase test \(DK\)](#) and [API 20E \(DK\)](#).

#### Oxidase test (e.g. Bactident Oxidase, Merck Cat. No. 1.13300.0001)

- a. Remove a single isolated, well-developed colony from the culture medium with a loop.
- b. Apply the colony to the reactive zone of the oxidase strip and distribute with the aid of the loop.
- c. After 20-60 seconds compare the test strip with the colour scale provided. If cytochrome c oxidase-positive bacteria are present the reactive zone exhibits a blue to purple colour. If cytochrome c oxidase-negative bacteria are present the reactive zone exhibits remains colourless.



**NOTE:** If using comparable test, please follow manufacturer directions.

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*Continued on next page*

## Detection of *Salmonella* spp. by cultivation, *Continued*

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### Verification (*continued*)

#### API Rapid 20E (a 5 hour test)

- a. Transfer 1-4 colonies to a "API NaCl 0.85%, 2 ml" vial (corresponding to McFarland 0.5), and mix carefully.
- b. Inoculate the API Rapid 20 E strip: With the same pipette, distribute the suspension into the tubes of the strip. To avoid the formation of bubbles at the base of the tube, tilt the strip slightly forwards and place the tip against the side of the cupule.
  - For the CIT test, add 2 drops of the suspension (app. 50 µl) to fill the tube and lower position of the cupule.
  - For the other tests, only fill the tubes (app. 50 µl per tube). The accuracy of the filling is very important.
  - For the underlined tests (LDC, ODC and URE) completely fill the cupule with mineral oil.
- c. Incubate the strip at 34-38°C for 4-4½ hours.
- d. Read the strips by referring to the reading table (in the package insert) and the picture below:
  - VP test (performed in a safety bench wearing protective gloves): add 1 drop of each of VP 1 and VP 2 reagents. Wait 5-10 minutes. A red color indicates a positive reaction.
  - IND test: add 1 drop of JAMES reagent. The reaction takes place immediately. A red colour indicates a positive reaction.



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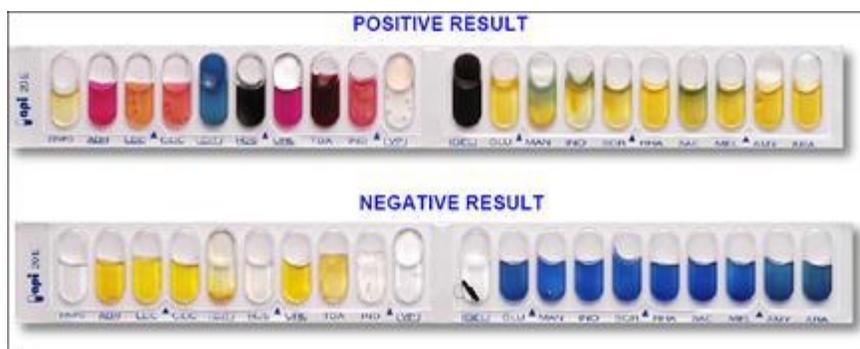
*Continued on next page*

## Detection of *Salmonella* spp. by cultivation, *Continued*

### Verification (continued)

#### API 20E (a 2 days test)

- a. Transfer 1 colony to a “API NaCl 0.85%, 5 ml” vial, and mix carefully.
- b. Inoculate the API 20 E strip: With the same pipette, distribute the suspension into the tubes of the strip. To avoid the formation of bubbles at the base of the tube, tilt the strip slightly forwards and place the tip against the side of the cupule.
  - For the CIT, VP and GEL tests, add 2 drops of the suspension (app. 50 µl) to fill the tube and lower position of the cupule.
  - For the other tests, only fill the tubes (app. 50 µl per tube). The accuracy of the filling is very important.
  - For the underlined tests (ADH, LDC, ODC, H<sub>2</sub>S and URE) completely fill the cupule with mineral oil.
- c. Incubate the strip at 34-38°C for 18-24 hours.
- d. Read the strips by referring to the reading table (in the package insert) and the picture below:
  - TDA test: add 1 drop of TDA reagent. A red / brown color indicates a positive reaction.
  - IND test: add 1 drop of JAMES reagent. The reaction takes place immediately. A red color indicates a positive reaction.
  - VP test (performed in a safety bench wearing protective gloves): add 1 drop of each of VP 1 and VP 2 reagents. Wait 5-10 minutes. A red colour indicates a positive reaction.



*Continued on next page*

## Detection of *Salmonella* spp. by cultivation, *Continued*

### API Identification

Read and determine ID using API webb:

Step	Action
1	Take out an API worksheet corresponding to the appropriate API strip (20E or Rapid 20E) and mark all the positive and negative results with a + or - .
2	Calculate the total score for each section of three tests on the API worksheet, only positives are tabulated. This will result in a 7 digit profile number.
3	Go to the website below, log-in, and choose the correct API test (either 20E or Rapid 20E): <a href="http://apiwebb.com">apiwebb™</a>
4	Enter the number for the appropriate group of 3 and hit confirm. Your Identification will appear. <i>NOTE:</i> For API 20E, a correct identification require $\geq 80\%$ similiarity. Contact responsible chemist if the ID score is $< 80\%$ .

**IMPORTANT:** In the LDC (Lysine decarboxylase) reaction it can be difficult to distinguish between Yellow/Negative and Orange/Positive. *Citrobacter braakii* may be wrongly identified as *Salmonella* based on this test. *C. Braakii* is Negative for LDC and *Salmonella* is Positive. If API identification is *Salmonella* Detected and LDC is Negative re-test and contact Responsible Scientist.

### Interpretation of results

If...	Then report result as...
No suspect colonies on XLD, Rambach or BS agar	<i>Salmonella</i> spp. not detected (ND)
Suspect colony on XLD, Rambach or BS agar <u>and</u> colony is oxidase positive	<i>Salmonella</i> spp. not detected (ND)
Suspect colonies on XLD, Rambach or BS agar <u>and</u> colony is oxidase negative <u>but</u> API Webb ID score is $< 80\%$	<i>Salmonella</i> spp. not detected (ND)
Suspect colonies on XLD, Rambach or BS agar <u>and</u> colony is oxidase negative <u>and</u> API Webb ID score is $\geq 80\%$	<i>Salmonella</i> spp. detected (DET)

*Continued on next page*

## Detection of *Salmonella* spp. by cultivation, *Continued*

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**Sensitivity and specificity** Sensitivity: 100%      Specificity: 100%  
*REFERENCE:* Luna No. [2008-20805-01](#).

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**Filing** All documentation should be filed in accordance with the local archiving SOP.

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**Contingencies** All deviations from this SOP should be discussed with the Method Responsible Scientist and should be documented.

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- References**
1. LUNA No. [2008-20805-01](#): Development and validation of two new methods for detection of *Salmonella* spp. in enzyme samples.
  2. LUNA No. [2012-02028-01](#): Hurtigere påvisning af *Salmonella* samt aflæsning af selektive plader opbevaret på køl. *In Danish*
  3. LUNA No. [2012-08403-01](#): Proficiency Testing Results – January 2012.

[Rambach agar](#), Merck

[XLD agar](#), Oxoid

[BS agar](#), Oxoid

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**Revision** Title of SOP changed. Detection of *Salmonella* spp. by PCR transferred to EB-SM-5075. Added that Brilliance *Salmonella* (BS) agar may be used instead of Rambach agar when selective enrichment is performed using RVs broth but not in combination with IMS (validated in LUNA No. [2008-20805-01](#)). Added amount of RVs broth used for secondary enrichment in the section “Media and reagents”. Minor editorial changes.

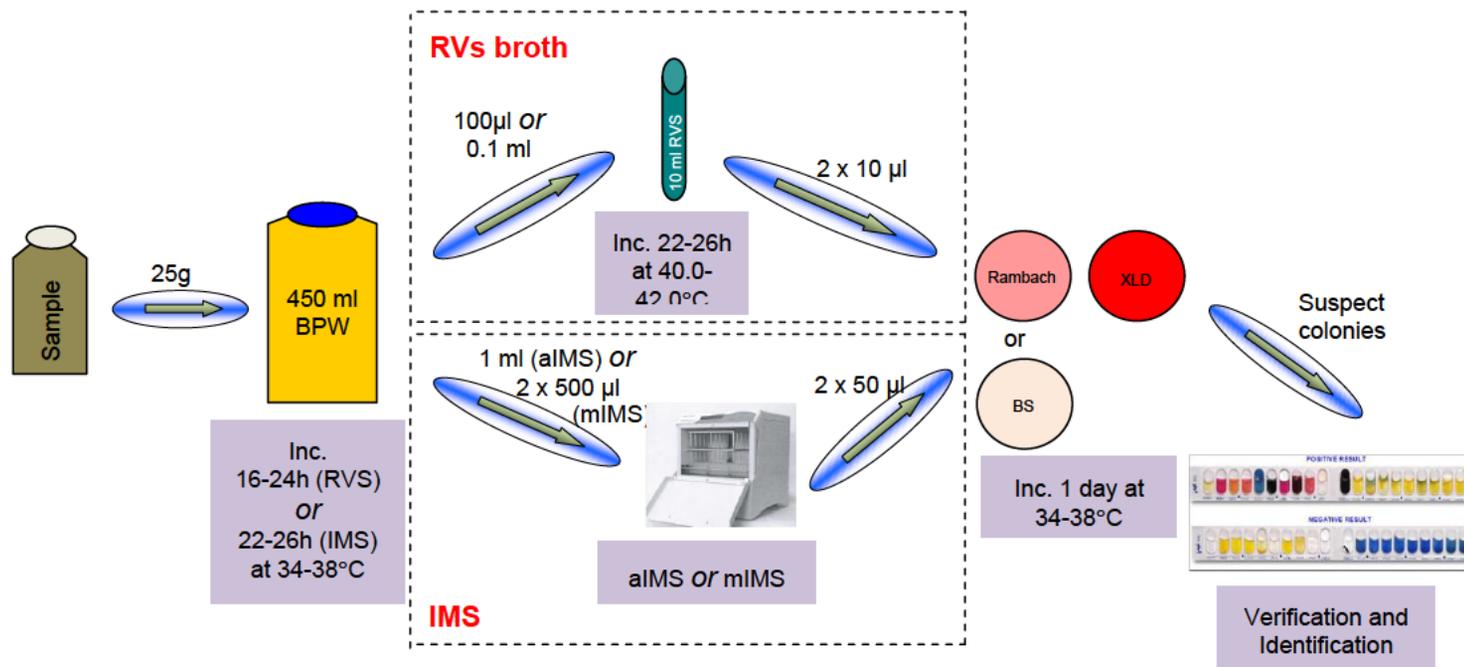
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## Detection of *Salmonella* spp. by cultivation, *Continued*

### Flow Chart

Flow chart of method. Ctrl + Click  to read section.



# Analytical method

Luna: 2014-05507-01

## **Detection of antimicrobial activity**

### **Principle**

Detection of Antimicrobial activity is based on the measurement of inhibition of bacterial growth under specific circumstances. The method is in accordance with JECFA (1992). Antimicrobial activity is measured as inhibition zone on agar plates with continues growth of 6 different bacteria.

### **Definition of units**

The result is stated as Antimicrobial activity detected (DET) or Antimicrobial activity not detected (ND)

### **Equipment, Reagents and Kits**

- Balance ( $\pm 0.1$  g)
- Sterile pipettes for transfer of 100  $\mu$ l, 1 ml and 10 ml
- Inoculation loops 1  $\mu$ l
- Paper discs, e.g. S&S Analytical Filter Papers No. 740-E (12.7 mm in diameter), autoclaved
- Bio Safety Cabinet, Class II
- Sterile gloves
- Refrigerator (2-8°C)
- Incubator (34-38°C)
- -80°C freezer (for cryo tubes)
- Ruler or Vernier gauge
- Petri dishes, 9 cm
- Tween buffer 4%
- Tryptone Soya agar (TSA), 90 ml in 250 ml Blue cap bottles
- Tryptone Soya agar plates, 9 cm with app. 15 ml agar (TSA)
- CASO broth in Blue cap bottles
- Ciprofloxacin discs (5  $\mu$ g or 10  $\mu$ g) (bought ready to use).

### **Microorganisms for test plates**

- *Staphylococcus aureus*, ATCC 6538
- *Escherichia coli*, ATCC 11229
- *Bacillus cereus*, ATCC 2
- *Bacillus circulans*, ATCC 4516
- *Streptococcus pyogenes*, ATCC 12344
- *Serratia marcescens*, ATCC 14041

### **Safety**

It is the responsibility of the laboratory leader that all personnel are aware of the correct handling of enzymes and reagents.

## Preparation of test plates

### Day 1

- Inoculate the 6 strains directly from cryo tube in separate 50 ml CASO broth and streak onto TSA agar.
- Incubate the CASO broth and TSA plates overnight at 34-38°C.

### Day 2

- Determine Total Viable Count in CASO broth by making a  $10^{-4}$  -  $10^{-5}$  dilutions of the CASO broth and spiral plate onto TSA agar. Incubate the plates overnight at 34-38°C.
- Check TSA plates from Day 1 for contaminants (may not be used if contaminated).
- Transfer 10 ml of the inoculated CASO broth to a 250 ml Blue cap bottle with 90 ml melted and cooled (app. 47 °C) TSA . Mix carefully. Note: Only 5 ml of the CASO broth containing *S. pyogenes*.
- Pour app. 10 ml of the TSA-microorganism mixture onto 10 TSA plates (containing app. 15 ml TSA). Distribute the TSA-microorganism mixture evenly on the surface of the TSA plates, and allow solidifying. Control the purity of the CASO broth by streaking out from the last drop of the bottle with a 1 µl inoculation loop onto the surface of one TSA plate and incubate at overnight at 34-38°C.
- Test plates have a shelf life of 1 month when stored at 2-8°C ,
- Verify the 6 different test plates by placing a Ciprofloxacin onto the middle of a test plate and incubate 1 day at 2-8°C followed by 1 day at 34-38°C.

### Day 3

- Check TSA plates from Day 2 CASO for contaminants (may not be used if contaminated).
- Count the number of colonies on the TSA spiral plates from Day 2, must be  $>10^6$  CFU

### Day 4

- Read inhibition zone on TSA plates with Ciprofloxacin from day 2 by measuring the diameter of the inhibition zone on each of the test plates using a ruler or a Vernier gauge. Each zone must be  $\geq 25$  mm.

## Test procedure

- Transfer 10 g of solid sample or 10 ml of liquid sample to 90 ml Tween buffer 4%. Immediately homogenize the sample by stirring or by shaking. Solid samples are homogenized on a magnetic stirrer for app. 20 minutes.
- Place a sterile paper disc on each of the different 6 test plates and inoculate paper disc with 100 µl  $10^{-1}$  sample dilution prepared. Up to 5 discs may be placed on each plate, making it possible to analyze 5 products per set of 6 test plates.
- Incubate the test plates 1 day at 2-8°C followed by 1 day at 34-38°C.
- Measure the diameter of the inhibition zone on each of the test plates using a ruler or a Vernier gauge.

## Interpretation of results

Test plates are interpreted accordingly to:

Is there...	with a zone measuring...	...the result is
0 inhibition zones	0 mm	Not detected (ND)
X inhibition zones	<16 mm	Not detected (ND)
1 inhibition zones	≥16 mm	Not detected (ND)
2 inhibition zones	≥16 mm	Not detected (ND)
3 inhibition zones	≥16 mm	Detected (DET)

If the result is Detected (DET) a remark is given on which of the test organisms that shows obvious antimicrobial activity in the sample and the size of the zone is stated. The Responsible Scientist is always contacted if a sample has antimicrobial activity.

If not all 6 different test plates are used f. ex. due to plate type not approved Day 4, not used test plates count as DET (f. ex. if 2 test plates are missing and 1 test plate has an inhibition zone ≥16 mm the result is DET)

For registration and GLP samples all 6 test plates need to be approved and used for analysis.

## Reference

Joint FAO/WHO Expert Committee on Food Additives (JECFA). Compendium of food additive specifications, Volume 1, Rome 1992, appendix A to annex 1.

## Handling of enzymes and chemicals

Enzymes and enzyme solutions should be handled in a fume hood or in closed containers. Avoid inappropriate handling of enzymes and enzyme solutions, which may result in aerosol/dust generation. Avoid inhalation of dust aerosols and contact with skin and eyes. Handling of chemicals and disposal of waste must be performed according to valid procedures.

## Validity

Valid from March 2014.

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**Novozymes A/S**  
Krogshøjvej 36  
2880 Bagsværd  
Danmark

[www.novozymes.com](http://www.novozymes.com)  
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*Novozymes is the world leader in bioinnovation. Together with customers across a broad array of industries we create tomorrow's industrial biosolutions, improving our customers' business, and the use of our planet's resources. Read more at [www.novozymes.com](http://www.novozymes.com).*

## Detection of production strains

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**Scope** All Novozymes QC laboratories involved in analysis of samples from Novozymes production and GLP studies.

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**Principle** **The production strain** is defined as the organism used for fermentation of a given Novozymes product. Agar media and incubation conditions used for detection of a specific production strain is listed in [BD 001-IN-000](#)

**The reference strain** is defined as an isolate of the production strain used in the laboratory as a reference during the analysis.

Strains not listed in [BD 001-IN-000](#) are detected according to specific **Analytical Directions** prepared and approved by the EB Method Responsible Scientist. Analytical Directions are typically used in connection with GLP studies.

When analyzing samples from Novozymes production, the detection is carried out by spread plating of 0.1 g or 0.1 ml of sample.

When analyzing samples from GLP studies, the detection is carried out by spread plating or enrichment of 1 g of sample acc. to the specific Analytical Direction.

Detection of morphologically typical colonies (compared with the reference strain) indicates the presence of the production strain.

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**Definition of units** When analyzing samples from Novozymes production, the result is stated as:

- DET (The productions strain detected in 0.1 g or 0.1 ml) *or*
- ND (The productions strain not detected in 0.1 g or 0.1 ml)

When analyzing samples from GLP studies, the result is stated as:

- DET (The productions strain detected in 1 g) *or*
- ND (The productions strain not detected in 1 g)

*IMPORTANT:* When detected, the app. number of production strain / g or ml is stated.

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**Samples** Novozymes products

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*Continued on next page*

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## Detection of production strains, *Continued*

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### Detection limit

The detection limit of this method is dependent on the sample volume and the dilution in use.

Sample volume	Size and number of agar plates	Dilution	Detection limit
1 ml, spread plate	14 cm (4 plates)	10 <sup>-1</sup>	10 colonies / g or ml
10 ml, spread plate	14 cm (40 plates)	10 <sup>-1</sup>	1 colonies / g or ml

---

### Equipment

Balance ( $\pm 0.1$  g)  
Magnetic stirrer  
Petri dishes (14 cm and 9 cm)  
Suitable sterile pipettes for transfer of 10 ml, 1 ml (4x0.25 ml) and 0.25 ml  
Sterile Drigalski spatula  
Incubator  
(relevant incubation temperatures are listed in [BD 001-IN-000](#))

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## Detection of production strains, *Continued*

### Media and reagents for Bacterial strains

Dilution buffer: Tween buffer 4%, 90 ml (If necessary, with a magnet) prepared acc. to [EB-ME-0052](#)

Agar media:

Abbreviation	Full name	Prepared acc. to EB Media direction (link)	Purpose
AT-2	AT-2 agar	<a href="#">EB-ME-0001</a>	Detection & verification
B-TSA	Basic Tryptic Soy Agar	<a href="#">EB-ME-0055</a>	Detection
B-TSA w.CAM	Basic Tryptic Soy Agar with or without Chloramphenicol (CAM) <i>NOTE: The addition of CAM is optional</i>	<a href="#">EB-ME-0056</a>	Detection
Schaeffers	Schaeffers agar	<a href="#">EB-ME-0036</a>	Verification
Sch.starch	Schaeffers agar with 1% starch	<a href="#">EB-ME-0037</a>	Verification
Skim milk	Tryptic Soy Agar with 1 % skim milk	<a href="#">EB-ME-0038</a>	Verification
TBX w.AMP	Chromocult®TBX agar + ampicillin (100 mg/l)	<a href="#">EB-ME-0066</a>	Detection
TSA	Tryptic Soy Agar	<a href="#">EB-ME-0041</a>	Detection
TSA w.CAM	Tryptic Soy Agar with or without Chloramphenicol (CAM) <i>NOTE: The addition of CAM is optional</i>	<a href="#">EB-ME-0057</a>	Detection
TSA w.kana	Tryptic Soy Agar with kanamycin	<a href="#">EB-ME-0058</a>	Detection

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## Detection of production strains, *Continued*

**Media and reagents for Fungal strains** Dilution buffer: Tween buffer 4%, 90 ml (If necessary, with a magnet) prepared acc. to [EB-ME-0052](#)  
Agar media:

Abbreviation	Full name	Prepared acc. to EB Media direction (link)	Purpose
Cove-T-2	Cove-T-2 agar	<a href="#">EB-ME-0013</a>	Detection & verification
DG-18	DG-18 agar	<a href="#">EB-ME-0017</a>	Verification
Phytate	Phytate agar	<a href="#">EB-ME-0028</a>	Verification
Sch.starch	Schaeffers agar with 1% starch	<a href="#">EB-ME-0037</a>	Verification
YPG	YPG agar with or without tetracycline <i>NOTE:</i> The addition of tetracycline is optional	<a href="#">EB-ME-0044</a>	Detection
YPSS	YPSS agar with or without tetracycline <i>NOTE:</i> The addition of tetracycline is optional	<a href="#">EB-ME-0045</a>	Detection
YSG	Yeast/Soy Peptone/Glucose	MSA-SUB-FS-0064	Verification

If verification on Schaeffers agar with starch is performed then Lugol's iodine solution (0.5%) is used. Lugol's solution is prepared acc. to [EB-ME-0021](#).

### Safety

It is the responsibility of the laboratory leader, that all personnel are aware of the correct handling of enzymes and reagents.

*Continued on next page*

## Detection of production strains, *Continued*

### Sample preparation

The samples are prepared as follows:

Step	Action
1	Transfer 10 g of solid sample or 10 ml of liquid sample to 90 ml Tween buffer 4%.
2	Immediately homogenize the sample by stirring or by shaking. Solid samples are homogenized on a magnetic stirrer for app. 20 minutes.

**IMPORTANT:** All enzyme products must be analyzed from a  $10^{-1}$  dilution due to possible inhibition of micro organisms in undiluted enzyme.

### Plating

Plating must be done within 15 minutes from end of homogenization. If this is not possible, the sample can be stored at 2-8°C for up to 4 hours.

**NOTE:** Relevant agar plates and incubation conditions (time and temperature) are listed in [BD 001-IN-000](#)

Step	Action
1	<p><b>NOTE:</b> Prepare the test plates:</p> <ul style="list-style-type: none"> <li>When analyzing samples from Novozymes production: Transfer 1 ml from the <math>10^{-1}</math> dilution onto the surface of 4 relevant agar plates (14 cm) with app. 0.25 ml on each plate.</li> <li>When analyzing samples from Tox batches (GLP): Analyse according to the relevant Analytical Direction.</li> </ul>
2	<p>Prepare the 2 positive control plates:</p> <ul style="list-style-type: none"> <li>Transfer 0.25 ml from the <math>10^{-1}</math> dilution onto the surface of 1 relevant agar plate (14 cm), and streak the bacteria reference strain or point inoculate the fungal production strain onto the inoculated plate.</li> <li>Streak the bacteria reference strain or point inoculate the fungal strain onto another agar plate (not inoculated with sample).</li> </ul>
3	Leave the plates on the table until the sample has been soaked into the agar.

*Continued on next page*

## Detection of production strains, *Continued*

---

### Reading

The colonies on the test-plates are compared morphologically with the colonies of the reference strain.

If ...	Then ...
No suspect colonies are observed on the test-plates ...	The test is ended and the result is stated as: <b>ND</b> (the production strain is Not Detected)
Suspect colonies are observed on the test-plates ...	The test is continued as described below (Verification).

*IMPORTANT:* The reference strain must grow on both of the two positive control plates. If not, the test is repeated.

---

### Verification

Suspect colonies from the test plates and the reference strain are streaked or point inoculated onto one or more of the agar plates (9 cm or 14 cm) listed in [BD 001-IN-000](#) (column "Verification"). Inoculation and reading of these agar media are described below. The plates are incubated as described in the column "Verification". If necessary, these media can be supplemented with other agar media, e.g. the agar medium used for the detection.

---

### AT-2 agar

Detection of pullulanase activity:

	Description
<b>Principle</b>	Pullulanase-producing strains degrade the amylopectin in the agar. As a result, blue zones (haloes) will surround the colonies of the isolate.
<b>Inoculation</b>	Point inoculation
<b>Reading</b>	Colonies of the isolate are compared morphologically with the colonies of the reference strain. The surface of the plates is carefully flooded with Lugol's solution (0.5%). Blue zones surrounding the colonies in a reddish-brown medium indicate pullulanase activity. <i>NOTE:</i> If the production strain produces amylase in addition to pullulanase, clear zones will surround the colony. Between the clear zone and the reddish-brown medium a narrow blue zone might be seen.

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*Continued on next page*

## Detection of production strains, *Continued*

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**Cove-T-2 agar** Detection of amdS-transformed fungi:

	Description
<b>Principle</b>	GMO strains transformed with the marker amdS grow well on the agar, while other strains appear with feeble or no growth.
<b>Inoculation</b>	Point inoculation.
<b>Reading</b>	<i>NOTE:</i> Colonies of the isolate are compared morphologically with the colonies of the reference strain. Vigorous growth on Cove-T-2 indicates presence of an amdS-transformed strain.

---

**DG-18 agar** Comparison of morphology of fungi:

	Description
<b>Principle</b>	DG-18 is a general growth medium for Fungi. The agar is used for comparison of morphology of fungal isolates with the reference strain.
<b>Inoculation</b>	Point inoculation
<b>Reading</b>	<i>NOTE:</i> Colonies of the isolate are compared morphologically with the colonies of the reference strain.

---

**Phytate agar** Detection of phytase activity:

	Description
<b>Principle</b>	Phytase-producing strains degrade phytate in the agar. As a result, clear zones (haloes) will surround the colonies of the isolate.
<b>Inoculation</b>	Point inoculation
<b>Reading</b>	<i>NOTE:</i> Colonies of the isolate are compared morphologically with the colonies of the reference strain. Before inoculation the plates are opaque. The presence of phytase activity is indicated by presence of clear zones (haloes) surrounding the colonies.

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*Continued on next page*

## Detection of production strains, *Continued*

### Schaeffers agar

Sporulation test (*Bacillus* spp.):

	Description
<b>Principle</b>	Schaeffers agar induces sporulation of wild type strains, but the production strains show no sporulation on Schaeffers agar after incubation for 2-3 days.
<b>Inoculation</b>	Point inoculation
<b>Reading</b>	<i>NOTE:</i> Colonies of the isolate are compared morphologically with the colonies of the reference strain. The colonies are examined by microscopy for sporulation. The production strain shows no sporulation after incubation for 2-3 days.

### Schaeffers starch agar

Detection of amylase activity (all isolates) and sporulation test (*Bacillus* spp.):

	Description
<b>Principle</b>	<u><i>Bacillus</i> spp.:</u> Schaeffers agar induces sporulation of wild type <i>Bacillus</i> strains, but the <i>Bacillus</i> production strains show no sporulation on Schaeffers agar after incubation for 2-3 days. <u><i>Bacillus</i> spp. &amp; Fungi:</u> Amylase producing strains degrade the starch in the agar. As a result, in clear zones (haloes) will surround the colonies of the isolate.
<b>Inoculation</b>	Point inoculation
<b>Reading</b>	Colonies of the isolate are compared morphologically with the colonies of the reference strain. <u><i>Bacillus</i> spp.:</u> The colonies are examined by microscopy for sporulation. The production strain shows no sporulation after incubation for 2-3 days. <u><i>Bacillus</i> spp. &amp; fungi:</u> The surface of the plates is carefully flooded with Lugol's solution (0.5%). Clear zones around the colonies in a blue (dark blue) indicates amylase activity.

*Continued on next page*

## Detection of production strains, *Continued*

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**Skim milk agar** Detection of proteolytic activity:

	Description
<b>Principle</b>	Protease-producing strains degrade the skim milk in the agar. As a result, clear zones (haloes) surround the colonies of the isolate.
<b>Inoculation</b>	Point inoculation
<b>Reading</b>	<i>NOTE:</i> Colonies of the isolate are compared morphologically with the colonies of the reference strain. Before inoculation the plates are opaque. Presence of clear zones (haloes) surrounding the colonies of the isolate after end of incubation indicate the presence of a proteolytic activity.

---

### Calculation

The result is stated on the basis of the number of typical colonies.

- No typical colonies: ND (Production strain not detected in 0.1 g or 0.1 ml)
- Typical colonies: DET (Production strain detected in 0.1 g or 0.1 ml).

If detected, the app. number of production strains / g or ml is stated.

*IMPORTANT:* If any production strain is detected, the Method Responsible Scientist is contacted immediately. In addition, QCC-cor is informed by mail.

---

### Accuracy and precision

The theoretical detection limit is:

- When analysing samples from Novozymes production:  
10 production strains / g or ml
  - When analysing samples from GLP studies:  
1 production strains / g
- 

### Archiving

All documentation should be archived in accordance with the local archiving SOP.

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*Continued on next page*

## Detection of production strains, *Continued*

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**Contingencies** All deviations from this SOP should be discussed with the Method Responsible Scientist and should be documented.

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**References** [BD 001-IN-000](#)

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**Revision** "EB – Productions Strain list" changed to [BD 001-IN-000](#)

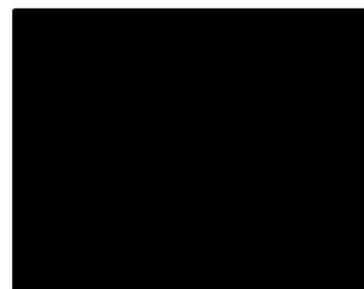
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<b>Document:</b>	Method UT015a	<b>No.:</b>	2.1
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<b>Title:</b>	Methods for Determination of Elements in Solid and Liquid Enzyme Samples and Samples of Polysaccharides by ICP-MS with Microwave-induced Sample Preparation	<b>Effective date:</b>	01.04.2014
		<b>Supersedes:</b>	2.0
		<b>To be revised:</b>	April 2017
<b>Prepared by:</b>	NB	<b>Approved by:</b>	EVJ
		<b>Date:</b>	01.04.2014

**Danish Technological Institute**  
Chemistry and Microbiology – Taastrup

18.03.2014



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**Area of application**

The method is applicable to solid and liquid enzyme samples and samples of polysaccharides for the ICP-MS determination of:

Ag, As, Bi, Cd, Co, Cu, Hg, Mo, Ni, Pb, Sb, Sn and Zn.

The method determines the total content of the specified elements in the stated matrices. The results are used in connection with product control.

The total heavy metal content given by  $\sum T$  comprises the elements Ag, As, Bi, Cd, Co, Cu, Hg, Mo, Ni, Pb, Sb and Sn.  $\sum T$  is reported as less than the sum of the product limits for the heavy metals stated in Table 1, where they cannot be detected in a sample. In cases where one or more heavy metals are measured in a concentration above the stated product limit(s), the measured value is included in the amount, which will then be a figure greater than 4.1 mg/kg.

The elements in Table 2 are not comprised by the accredited method, but are continually analysed with the accredited elements.

**Table 1. Detection and product limits**

Element	DL accr. no. 90 ppm (mg/kg)	Product limit ppm (mg/kg)	DTI product limit ppm (mg/kg)
Ag	0.01	-	0.5
As	0.02	3	0.3
Bi	0.01	-	0.5
Cd	0.01	0.5	0.05
Co	0.01	-	-
Cu	0.02	1	0.5
Hg	0.01	0.5	0.05
Mo	0.02	-	0.1
Ni	0.04	1	0.5
Pb	0.02	5	0.5
Sb	0.02	-	0.5
Sn	0.01	-	0.5
Total heavy metal content, $\sum T$		30	4.1

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**Table 2. Non-accredited elements**

Element	DL ppm (mg/kg)	Product limit ppm (mg/kg)	DTI product limit ppm (mg/kg)
Cr	0.01	-	0.1
Se	0.02	-	0.2
Zn	0.1	-	0.5

**Table 3. Measuring capability****Measuring  
capability**

Parameter	Upper meas. limit mg/kg	Quanti-zation limit mg/kg	Detection limit mg/kg	%RS D
Ag	5	0.5	0.01	15
As	10	0.1	0.02	15
Bi	5	0.5	0.01	15
Cd	5	0.05	0.01	17
Co	5	0.1	0.01	15
Cu	10	0.1	0.02	30
Hg	5	0.03	0.01	16
Mo	10	0.1	0.02	16
Ni	10	0.1	0.04	22
Pb	100	0.5	0.02	17
Sb	5	0.5	0.02	20
Sn	5	0.5	0.01	20

See annex 2 for uncertainty budgets.

**Principle**

After the dry or liquid product has been weighed, it is destroyed with nitric acid in closed PFA autoclaves by microwave-induced heating. The destroyed material is then diluted, filtered and analysed by ICP-MS for content of Ag, As, Bi, Cd, Co, Cu, Hg, Mo, Ni, Pb, Sb, Sn and Zn.

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<b>Laboratory equipment</b>	<p>Weighing boats, plastic disposable syringes</p> <p>Analytical balance <math>\pm 0.1</math> mg</p> <p>Microwave oven</p> <p>100-150 ml PFA autoclaves</p> <p>Funnels (d = 40 mm with thick stalk)</p> <p>Filter paper Munktell OOK (d = 110 mm, d = 90 mm)</p> <p>Volumetric flasks, 50 and 100 ml</p> <p>Polyethylene vessels, 50 and 100 ml (e.g. Kautex, Nalgene)</p> <p>Fine pipettes</p> <p>Autosampler glass, 15 and 50 ml (e.g. Hounisen PP)</p>
<b>Reagents</b>	<p>Demineralised water Millipore Q-plus concentrated nitric acid (HNO<sub>3</sub> 14 M) subboiling prepared from Merck p.a.</p> <p>1.75 M and 2.8 M HNO<sub>3</sub> from concentrated nitric acid (Subboiling). See Instruction T1801c for preparation</p>
<b>Standards</b>	<p>Standards of 0, 1, 5 and 10 ng/ml of the elements in 2.8 M HNO<sub>3</sub> prepared as per Instruction T1801c, although standards of 0, 0.5, 1 and 2 ng/ml are used for Hg.</p>
<b>Calibration control</b>	<p>Use a "Control I" of 10 ng/ml for calibration control solution and a "Control IV" of 50 ng/ml for linearity, likewise prepared as described in Instruction T1801c. For Hg use a control Hg_1 of 1.0 ng/ml prepared as per Instruction T1801c. The operational acceptance criterion for control I, IV and Hg_1 are within <math>\pm 10\%</math> of the control value; if that is not met, the person responsible for the analysis should be consulted. Control I and Hg_1 are recorded in control charts. See Instruction T 1804.</p>
<b>Control samples</b>	<p>Merck ICP multielement standard solution VI, prepared as the sample, is included as control sample for preparation. The operational acceptance criterion for Merck VI is <math>\pm 10\%</math> of the control value; if that is not met, the person responsible for the analysis should be consulted.</p> <p>Merck VI is further recorded in control charts. See Instruction T 1804.</p>

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**Sample handling** Solid samples are stored at room temperature.

Liquid samples are stored in a refrigerator at  $< 5^{\circ}\text{C}$  until the time of analysis.

Samples received frozen are stored in a freezer until the time of analysis and then they are defrosted in a refrigerator.

**Preparing equipment**

**Cleaning**

The PFA autoclaves are cleaned with 20 ml conc.  $\text{HNO}_3$  per vessel by microwaving for 20 minutes at 100% and then they are rinsed with demineralised water.

**Preparing samples**

**Solid samples**

$0.5 \pm 0.1$  g of sample is weighed to 4 decimal places in a weighing boat and transferred to a PFA autoclave, and then the weigh boat is reweighed. When weighing highly viscous enzyme samples, a disposable syringe can be used with advantage as a weighing bottle.

The sample is suspended in 20 ml 7 M  $\text{HNO}_3$ .

The autoclave is closed, placed in the carousel and microwaved for 35 minutes at 630 W with regulation to max.  $230^{\circ}\text{C}$  and 20 bar in accordance with the instructions for use of the microwave oven.

After cooling the autoclaves are opened.

The contents are filtered into a 50 ml acid-rinsed volumetric flask with demineralised water and diluted to volume with demineralised water. The filtrate is stored in a polyethylene vessel.

**Liquid samples**

1 ± 0.5 g of sample is weighed to 4 decimal places in a weighing boat and transferred to a PFA autoclave, and then the weighing boat is reweighed. When weighing highly viscous enzyme samples, a disposable syringe can be used with advantage as a weighing bottle.

The sample is suspended in 20 ml 7 M HNO<sub>3</sub>.

The autoclave is closed, placed in the carousel and microwaved for 35 minutes at 630 W with regulation to max. 230°C and 20 bar in accordance with the instructions for use of the microwave oven.

After cooling the autoclaves are opened.

The contents are filtered into a 50 ml volumetric flask with demineralised water and diluted to volume with demineralised water. The filtrate is stored in a polyethylene vessel.

The analysis for heavy metals (Ag, As, Bi, Cd, Co, Cu, Hg, Mo, Ni, Pb, Sb, Sn and Zn) is carried out as duplicate determinations.

At least 2 blind samples are prepared accordingly for each sample series.

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**Analysis**

The analysis is performed by ICP-MS (Inductively Coupled Plasma Mass Spectrometry) in CCT mode with He as collision gas using external standards in 2.8 M HNO<sub>3</sub>, and continuously adding the internal standards in 0.14 M HNO<sub>3</sub> to the carrier solution.

Typical instrument parameters are stated in Tables 4 and 5.

A calibration blank of 2.8 M HNO<sub>3</sub> is prepared.

The system is rinsed with 1.75 M HNO<sub>3</sub> (carrier).

The autosampler probe is rinsed with 1.75 M HNO<sub>3</sub> (from Subboiling).

An example of a routine analysis set-up is shown in Annex 1. It will typically include the calibration blank, a standard series, quantification limit controls at 2 levels, e.g., 0.1 and 0.5 ng/ml, Control I and IV, an Hg control, and a double-determination on a control sample (Merch VI) that is entered on the control chart.

After every 10 – 12 samples, or on completion of a run, a calibration blank and controls I and Hg\_1 are analysed.

The controls should not deviate more than stated under 'calibration control'.

%RSD of the measurements of the quantification limit control 0.1 ng/ml should be less than 20 %, if this is not the case, the requirement shall be fulfilled for the quantification limit control 0.5 ng/ml.

See Table 1 for detection limits.

An automatic dilution factor should never be used.

Generally, the sample is diluted at concentrations of the analysis subject greater than 50 ng/ml. Alternatively, the sample is re-analysed by ICP-AES for these parameters.

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A tox batch sample is reanalysed when %RSD is larger than 20% for a double determination of an element, when the concentration is above the quantification limit of the method. All valid subresults are reported. This applies only for tox batch/GLP samples.

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**Table 4. Spectrometer parameters**

Element	Mass	Dwell time	CCT/He	Resolution	Notes
Ag	107	0.01	KED	H	
As	75	0.04	KED	N	
Bi	209	0.01	KED	H	
Cd	111	0.04	KED	N	
Co	59	0.01	KED	N	
Cr	52	0.01	KED	N	*
Cu	65	0.01	KED	N	
Ge	72	0.01	KED	N	IS
Hg	202	0.05	KED	N	
Mo	98	0.01	KED	N	
Ni	60	0.01	KED	N	
Pb	208	0.01	KED	H	
Re	187	0.01	KED	N	IS
Rh	103	0.01	KED	N	IS
Sb	121	0.01	KED	H	
Se	77	0.04	KED	N	*
Sn	120	0.01	KED	H	
Zn	66	0.01	KED	N	*

IS denotes internal standards.

CCT with He as collision gas and with KED

Resolution: N = normal resolution, H = high resolution

\* denotes that elements are not covered by the accreditation.

**Table 5. Plasma parameters**

Plasma flow (l/min)	15
Neb. flow (l/min)	0.9-1.1
Aux. flow (l/min)	0.8
CCT I (ml/min.)	4.8
RF power (W)	1550
Pump (rpm)	40
Main runs	3
Number of sweeps	15

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**Calculation**                      **Data processing and calculation of results**

Data from the ICP-MS software is transferred via the same software to excel format for further calculations.

**Calculation principle:**

The concentration in the sample,  $C_{\text{sample}}$ , is calculated as follows:

$$C_{\text{sample}} = (V \cdot (F \cdot C_{\text{measured}} - C_{\text{blank}})) / m_{\text{sample}}$$

where

$C_{\text{measured}}$  is the concentration in the measuring solution

$C_{\text{blank}}$  is the concentration in the blind sample. If  $C_{\text{blank}}$  is < the detection limit the value 0 is used

F is the dilution factor (normally 1)

V is 50 ml  $m_{\text{sample}}$  is the amount of weighed sample.

If the result is stated on dry matter basis,  $C_{\text{sample}}$  must be corrected for per cent dry matter (%DM).

$$C_{\text{sample}} = (100 \times C_{\text{sample}}) / \%DM$$

**Reporting**

Reports on liquid and solid samples and samples of polysaccharides are reported directly on the analysis requisition forms and that are returned by mail. The following information must be stated on the requisition form:

- Date of receipt/initials.
- Samples ID no. of the Laboratory for Chemistry and Microbiology.
- Date/initials.
- Results for the parameters selected by the person placing the order.

After mailing, the assignment is considered to have been reported.

For Toxbatch/GLP samples the analysis is considered to be finished, when the results have been mailed whereupon the sample shall be disposed.

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In the case of tox batch samples (GLP assignments), an authorised written report of the analysis is sent with full documentation in the form of an annex.

The annexes comprise:

- 1) Registration slip.
- 2) Weighing chart with weight ID no.  
Forms for preparation of intermediate dilutions, standards incl. standards for Hg and for control I and Hg\_control.  
Sample List, Calibration Curves, calculations and Raw Data all in excel format.  
On the first page of the printed raw data, the instrument ID no. is specified, e.g., T-1.0024 for Thermo iCAP Q. The raw data sheets are clipped together or collected in a plastic cover.
- 3) Instrument Parameter Settings.

All documents are stamped on the first page with the studio number.

## Safety

### Nitric acid HNO<sub>3</sub> conc.

Hazard symbols: O (oxidising) + C (corrosive).

R/S phrases: R8-35 S 23-26-36

Etching hazard

Highly inflammable when in contact with flammable material

Irritating to respiratory system

In case of contact with eyes, rinse immediately with plenty of water and contact a doctor

Wear special protective clothing and protective gloves

In case of accident or if you feel unwell, seek medical advice immediately (show the label).

Spills: Absorb with ABSOL. Strong ventilation.

Precautions: Work with nitric acid should be carried out in a fume hood, wearing gloves and safety goggles.

See directions for use.

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Especially when weighing powdery enzyme samples wear a mask and safety gloves. See reference 5 on handling enzyme samples.

**Literature**

1. Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma Mass Spectrometry. U.S. Environmental Protection Agency. Method 200.8, Revision 4.4, April 1991.
2. Users Manual  
Thermo Fisher Scientific, iCAP Q Operating Manual rev. C1288090
3. Report 17661, 21.12.1995:  
"Comparative analysis of 9 granulated enzyme samples and of 11 liquid enzyme samples for element contents by FI-ICP-MS and ICP-AES".
4. "Enzymes and you – a guide for laboratory workers".  
Novo Nordisk A/S.

**Annex 1**

Sample List

**Annex 2**

Uncertainty budgets

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### Correction log

Correction page no.	Previous version	New version
Page 2	3.0 mg/kg	4.1 mg/kg
Page 2	Table 1- Total heavy metal content 40	Table 1- Total heavy metal content 30
Page 4	"Control II" of 250 ng/ml	"Control IV" of 50 ng/ml for linearity
Page 4	Control sample	Control sample for preparation
Page 7	e.g. 0.1 and 0.5 ng/ml, Control I and II	e.g. 0.1 og 0.5 ng/ml, Control I and IV
Page 7	Detection limit controls	Quantification limit controls
Page 7		%RSD of the measurements of the quantification ..
Page 7	greater than 500 ng/ml	greater than 50 ng/ml
Page 8		in CCT mode with He as collision gas
Page 9	Table 4	Table 4, changed and added parameters
Page 9	Table 5	Table 5, changed and added parameters
Page 10	Data from ICP-MS software is transferred to external.....	Data from the ICP-MS software is transferred to excel ...
Page 10		For Toxbatch/GLP samples....
Page 11	Sample Batch Report, Dataset Report, calculations and Raw Data	Sample List, Calibration Curves, calculations and Raw Data all in excel-format
Page 12	T 1.0012 for PE Sciex Elan 5000	T 1.0012 deleted
Page 12	Users Manual ELAN 5000 ....	Deleted
Page 12		Users Manual Thermo Fisher Scientific, iCAP Q...

## Appendix 4

### Documentation regarding the manufacturing process

1. Statement on compliance of Good Manufacturing Practices, Food
2. ISO 9001:2008 certificate

To Whom It May Concern

March 18, 2013

Statement no. 401.13

### Statement on Good Manufacturing Practice - GMP

- general description of production, control and hygiene

Novozymes A/S is a manufacturer of enzymes used in the food industry. We hereby certify that:

The products are produced according to good manufacturing practices for manufacturing, packing, or holding human food in order to prevent serious food hazards. Furthermore, our documented quality system is ISO 9001<sup>1</sup> certified by DS Certificering, accredited by DANAK. The quality system includes:

- Production operations are conducted in accordance with adequate sanitation principles.
- HACCP plan. Critical control points (CCPs) are identified and controlled, and the products are released if in compliance with these requirements.
- Critical measuring equipment is identified and calibrated at regular intervals.
- Instructions on cleaning of equipment, utensils and rooms are established and cleaning is documented.
- The personnel is trained in hygienic practices in order to prevent contamination of products and equipment.
- The personnel is trained in the quality system.
- The buildings and equipment are monitored periodically with special reference to maintenance.
- The production of our food enzymes complies with EC regulation 852/2004/EC, including amendments, on *the hygiene of foodstuffs*.
- The packaging materials used for our food enzyme products comply with EC regulation 1935/2004/EC, and related legislation including amendments on materials and articles intended to come into contact with foodstuffs.
- The production is under control of and inspected by the authorities according to EC regulation 882/2004/EC, including amendments, on *the official control of foodstuffs* as interpreted and implemented in Danish legislation.

<sup>1</sup>The scope of the 9001 certificate is: Development, Production and Sales of Biopolymers and Industrial Enzymes.

**BUREAU VERITAS**  
Certification



## Certification

Awarded to

**Novozymes A/S**

*Sites as to attached appendix*

**Bureau Veritas Certification certifies that the Management System of the above organisation has been audited and found to be in accordance with the requirements of the Management System standards detailed below.**

STANDARD

**ISO 9001:2008**

SCOPE OF SUPPLY

**Development, production and sales of industrial enzymes.**

*Original approval date:* 25-03-1996

*Subject to the continued satisfactory operation of the organisation's Management System, this certificate is valid until:* 25-03-2015

*To check the validity of this certificate, please call: (+45) 77 311 000.*

*Further clarification regarding the scope of this certificate and the applicability of the system requirements may be obtained by consulting the organisation.*

*Certificate Number:* DK003201-2

*Date:* 06-06-2013



Certification body address: Brandon House, 180 Borough High Street, London SE1 1LB, UK  
Certification office: Oldenborggade 1B, DK-7000 Fredencia

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## Appendix 5

### Safety documentation

FÈ Ú^quence homology of serine protease from S10-34zEK4 to known toxins and allergen analysis of serine protease from S10-34zEK4.

Novozymes Report No.: 2011 -€í î €G

G Serine endopeptidase, batch PPA26797: Summary of toxicity data

Novozymes Report No.: 2013-03986.

H Serine endopeptidase, PPA 26797: Test for mutagenic activity with strains of *Salmonella typhimurium* and *Escherichia coli*.

Novozymes Study No.: 20078045.

Novozymes Report no.: 2007-38794.

I . Serine Endopeptidase, PPA 26797: Induction of chromosome aberrations in cultured human peripheral blood lymphocytes.

Covance Study No.: 1974/62.

Novozymes Reference No.: 2007-6030.

Í . Serine endopeptidase, PPA 26797, a 13-week Oral (Gavage) Toxicity Study in Rats.

LAB Research, Scantox Study No.: 66063.

Novozymes Reference No.: 2007-6029.

Sequence homology of Protease from S10-34zEK4 to known  
toxins  
and  
Allergen analysis of Protease from S10-34zEK4

Esben Friis  
LUNA# 2014-07602-01

May 15, 2014

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G.2 35% or larger identity over any 80 amino acid window (with scaling) . . . . .	98
G.3 Identities calculated from Needleman-Wuncsh alignment . . . . .	98

# 1 Sequence homology of Protease from S10-34zEK4 to known toxins

## Uniprot database

Protein sequences that contain the word *toxin* in the description field were extracted from UNIPROT (Database date: 15-Jul-2013). This database contains entries from SWISSPROT and TREMBL. 50030 entries were found. Each of the sequences was placed in its uniquely named Fasta file. The Protease from S10-34zEK4 sequence was placed in a separate file "S10-34zEK4.fasta". The awk script in appendix A was used to invoke the sequence alignment program ClustalW 2.0.10 to align each sequence to Protease from S10-34zEK4. A summary file containing the length of each sequence and number of identical residues is also created. From this, the identity percentage to the Protease from S10-34zEK4 sequence or the compared toxin sequence is calculated, whichever is longest. This is chosen because the toxin sequences have many different lengths, both much shorter and much longer than the Protease from S10-34zEK4 sequence. By always using the longest sequence, artificial high scores from very short or very long toxins are avoided. The largest homology encountered was 22.3%, indicating that the homology to any toxin sequence in this databas is indeed random and very low. The results are shown in appendix B.

## 2 Allergen analysis of Protease from S10-34zEK4

### Allergen Databases

The EFSA scientific opinion [1] recommend that searches are done in more than one allergen database, to ensure that as many known allergens as possible are considered. In this case, all available allergen sequences were downloaded from the following databases:

- <http://allergenonline.org>. This is the home page of the The Food Allergy Research and Resource Program (FARRP) allergen protein database. The present report use data downloaded 15-Jul-2013. Appendix D shows a list. A few of the entries were omitted, due to wrong accession codes, unpublished sequences or other errors, see appendix D.1.
- <http://www.allergen.org>. This is the official site for the systematic allergen nomenclature that is approved by the World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee. The present report use data downloaded 15-Jul-2013. Appendix E shows a list. A few of the entries were omitted, due to wrong accession codes, unpublished sequences or other errors, see appendix E.1.

### Analyses

1. more than 35% identity in the amino acid sequence of the expressed protein (i.e.without the leader sequence, if any), using a window of 80 amino acids and a suitable gap penalty (using Clustal-type alignment programs or equivalent alignment programs). This is one of the recommended test methods of the EFSA scientific opinion [1], and also of the earlier publication from the FAO/WHO Expert group [2]. The queries were done using Fasta 3.4, using the scripts in appendix C
2. same as item 1, but with scaling enabled. In this way, matches with high identity, but over windows shorter than 80 amino acids can be identified. For example a match with 50% identity over 60 amino acids would still have enough identical amino acids to exceed the 35% threshold over 80 amino acids:  $60 \cdot 0.50/80 = 0.375 = 37.5\%$ .
3. Alignment of Protease from S10-34zEK4 to each of the allergens, and identify hits with more than 35% identity over the full length of the alignment. These queries were performed using the global alignment "needle", which is an implementation of the Needleman-Wunsch global alignment algorithm [3] in the program package EMBOSS [4].

The two first are in compliance with the recommendations in the EFSA scientific opinion [1]. The latter adds some more detailed information for hits identified by the two first methods.

## Results

**Database: allergenonline.org**

### **35% identity over 80 amino acids**

The following allergens had one or more matches using the method described in item 1 above (see appendix F for a complete list).

(No hits found)

### **35% identity over 80 amino acids with scaling**

The following allergens had one or more matches using the method described in item 2 above (see appendix F for a complete list).

(No hits found)

### **Identity over full length**

All allergens with more than 10% sequence identity to Protease from S10-34zEK4 are shown in appendix F.3. The identities to the allergens identified by the 35% identity over 80 amino acids method are shown below.

(No hits found)

**Database: allergen.org**

### **35% identity over 80 amino acids**

The following allergens had one or more matches using the method described in item 1 above (see appendix G for a complete list).

(No hits found)

### **35% identity over 80 amino acids with scaling**

The following allergens had one or more matches using the method described in item 2 above (see appendix G for a complete list).

(No hits found)

### Identity over full length

All allergens with more than 10% sequence identity to Protease from S10-34zEK4 are shown in appendix G.3. The identities to the allergens identified by the 35% identity over 80 amino acids method are shown below.

(No hits found)

### Conclusion

No significant homology was found between Protease from S10-34zEK4 and any of the allergens in the databases mentioned above.

### References

- [1] Scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA panel on genetically Modified Organisms (GMO panel). European Food Safety Authority (EFSA), Parma 2010. (The document may be downloaded from <http://www.efsa.europa.eu/en/scdocs/scdoc/1700.htm>)
- [2] Evaluation of Allergenicity of Genetically Modified Foods (Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology 22-25 January 2001), Food and Agriculture Organization of the United Nations (FAO), Rome 2001. [http://www.who.int/foodsafety/publications/biotech/ec\\_jan2001/en/](http://www.who.int/foodsafety/publications/biotech/ec_jan2001/en/)
- [3] Needleman, S. B. and Wunsch, C. D. (1970) *J. Mol. Biol.* **48**, p 443-453.
- [4] Rice, P., Longden, I. and Bleasby, A. (2000): "EMBOSS: The European Molecular Biology Open Software Suite" *Trends in Genetics* **16**, No 6. p 276-277

## A Scripts for toxin homology search

Awk scripts for alignment of sequences to the Protease from S10-34zEK4 sequence and calculation of sequence lengths and identities. First the script used to run the alignments. The script is stored in a file called "runaligns".

```
#!/bin/tcsh
cat NZYM-RH.fasta $1 >tmp.txt
clustalw tmp.txt
grep -v ">" $1 | gawk '{printf "%s",$0} END {printf "\n"}' | wc | \
  gawk '{print $3-1}' > $1.len
cat tmp.aln | gawk '{printf "%s",$0} END {printf "\n"}' | \
  sed 's/[^\*]//g' | wc | gawk '{print $3-1}' > $1.idt
echo $1 | gawk '{printf "%s ",$0}' >> summary
cat $1.len $1.idt | gawk '{printf "%s ",$0} END {printf "\n"}' >>summary
mv tmp.aln $1.out
```

Before start, the file "summary" must be deleted. The analysis is automatically done for all .fasta files in the current directory (and subdirectories, if present) by the command:

```
find . -name "*.fasta" -exec runaligns {} \;
```

Afterwards the sequence length and identity information can be found in the file summary. This file is processed through the following Python script, which calculates the percentages as described in the text.

```
#!/usr/bin/python
import string,commands

compare_length = 188
data = []

f = open ("summary","r")

buffer = "XX"
i=0

while buffer != "":
    buffer = f.readline()
    if buffer != "":
        data.append(string.split(buffer))
        data[i][1] = int(data[i][1])
        data[i][2] = int(data[i][2])

        i = i+1
f.close()
```

```
for i in range(len(data)):
    fullname = commands.getoutput("grep "+string.upper(data[i][0][2:-6])+" description.txt")
    percentid = 100.0*float(data[i][2])/float(max(data[i][1],compare_length))
    if (percentid >= 10.0):
        printlist = [data[i][0][2:-6], data[i][1], data[i][2], \
                    percentid, \
                    fullname[18:83] ]
        print '%-13s %4d %4d %5.1f  %-60s' % tuple(printlist)
```

## B Toxin homology results

UNIPROT entries, that contain the word "toxin", but not "fragment" in the description field and their identity to Protease from S10-34zEK4. The columns are

1. sequence database accession number
2. sequence length
3. number of identical residues after alignment to Protease from S10-34zEK4
4. percent identity compared to Protease from S10-34zEK4 or the sequence, whichever is longest.
5. sequence description

Matches  $\geq 10\%$  are shown

## C Scripts for allergen analysis

### Script for making the search for identity over a window

```
#!/bin/csh
#
# USAGE: windowmatch <query sequence> <library> <windowlength> <cutoff> <raw fasta output>
# e.g. windowmatch BG025.fasta allergenonline.fasta 80 35.0 ../rawfasta.txt
#
awk -v window=$3 -f ./makewindows.awk $1
find . -name "window$3_*.fasta" -exec /z/linux/fasta/fasta34 -Q -b 100000 -d 100000 -w 100 {} \
  $2 2 \; | tee $5 | grep -A 2 ">>" \
  | awk -v window=$3 -v threshold=$4 '/^>>/ {name= substr($1,3,200); getline; getline; percent=gensub("%", "", "g", $4); \
  overlap= int($9); if (1.0*percent >= 1.0*threshold && 1*overlap >= 1*window) \
  {printf "%s\t%3.1f%% identity i %2d aa overlap.\n", \
  name, percent, overlap}}' | sort -r -n --key=10
```

The script is invoked by the following command, where parameter 2 is the length of the window, and parameter 3 is the identity threshold:

```
windowmatch S10-34zEK4.fasta allergenonline.fasta 80 35.0 >
allergenonline_window80_result.txt
```

and

```
windowmatch S10-34zEK4.fasta allergen.org.fasta 80 35.0 >
allergenorg_window80_result.txt
```

### Script for making the search for scaled identity over a window

```
#!/bin/csh
#
# USAGE: windowmatch_scale <query sequence> <Library> <windowlength> <cutoff> <raw fasta output>
# e.g. windowmatch BG025.fasta allergenonline.fasta 80 35.0 myfastaoutput.txt
#
awk -v window=$3 -f ./makewindows.awk $1
find . -name "window80_*.fasta" -exec /z/linux/fasta/fasta34 -Q -b 100000 -d 100000 -w 100 {} \
  $2 2 \; | tee $5 | grep -A 2 ">>" \
  | awk -v window=$3 -v threshold=$4 '/^>>/ {name= substr($1,3,200); getline; getline; percent=$4; \
  overlap= int($9); newpercent=(1.0*percent*overlap)/(1.0*window); if (newpercent >= 1.0*threshold && overlap < window) \
  {printf "%s\t%3.1f%% identity i %2d aa overlap, scaled to %3.1f%% identity i %d aa overlap\n", \
  name, percent, overlap, newpercent, window }}' | sort -r -n --key=10
```

The script is invoked by the following command, where parameter 2 is the length of the window,

and parameter 3 is the identity threshold. This script allows identification of matches with higher identity over shorter windows than 80 amino acids. For example a match with 50% identity over 60 amino acids would still have enough identical amino acids to exceed the 35% threshold over 80 amino acids:  $60 \cdot 0.50/80 = 0.375 = 37.5\%$ .

```
windowmatch_scale S10-34zEK4.fasta allergenonline.fasta 80 35.0 >
allergenonline_window80_result_scale.txt
```

and

```
windowmatch_scale S10-34zEK4.fasta allergen.org.fasta 80 35.0 >
allergenorg_window80_result_scale.txt
```

## Common awk script used by the two previous scripts

The file is named `makewindows.awk`

```
BEGIN { seq=""
  if (window < 1)
    window = 6
}
{
  if (substr($0,1,1) != ">")
  {
    gsub("[^A-Za-z]", "")
    seq = sprintf("%s%s", seq, $0)
  }
}
END {
  for (i=1; i<length(seq)-window+2; i++)
  {
    filename = sprintf ("window%d_%04d.fasta", window, i)
    printf ">window%d_%04d\n", window, i > filename
    printf "%s\n", substr(seq,i>window) > filename
  }
}
```

## Script for making the Needleman-Wunsch alignment and comparison

```
#!/bin/csh
#
# USAGE: fullmatch <query sequence> <library> <cutoff> <raw needle output>
# e.g. fullmatch BG025.fasta 35.0 ../rawneedle.txt
#
```

```
needle -asequence $1 -bsequence $2 \  
-gapopen 10.0 -gapextend 0.5 -outfile /dev/stdout \  
| tee $4 | awk -v threshold=$3 '2:/{name = substr($3,1,80) } \  
/Identity/{ matches = $3; percent = strtonum(gensub("\\(", "", 1, $NF)); \  
if (percent >= threshold) {printf "%-80s %-10s = %5.1f%%\n", \  
name, matches, percent } } ' | sort -r -n --key=4
```

The script is invoked by the following command, where parameter 1 is the identity threshold:

```
fullmatch S10-34zEK4.fasta allergenonline.fasta 10.0 >  
allergenonline_fullresult.txt
```

and

```
fullmatch S10-34zEK4.fasta allergen.org.fasta 10.0 >  
allergenorg_fullresult.txt
```

## D List of allergens from allergenonline

List of allergens that have been tested by the EFSA scientific opinion recommended allergen analysis described in section 2. The sequences were downloaded via <http://allergenonline.org>.

Count	Species	Common	IUIS Allergen	Type	Group	Length	GI#	FirstVersion
1	Acarus siro	Mite	Unassigned	Aero Mite	Acarus Aca s 13	131	118638268	9
2	Actinidia chinensis	Kiwi	Unassigned	Food Plant	Actinidia Act c 1 Act d 1	380	190358935	9
3	Actinidia deliciosa	Kiwi	Unassigned	Food Plant	Actinidia Act c 1 Act d 1	380	15984	7
4	Actinidia deliciosa	Kiwi	Unassigned	Food Plant	Actinidia Act c 1 Act d 1	380	166317	7
5	Actinidia deliciosa	Kiwi	Unassigned	Food Plant	Actinidia Act c 1 Act d 1	380	193806686	12
6	Actinidia chinensis	Kiwi	Unassigned	Food Plant	Actinidia Act c 8 Act d 8 PR-10	159	281552896	11
7	Actinidia deliciosa	Kiwi	Unassigned	Food Plant	Actinidia Act c 8 Act d 8 PR-10	157	281552898	11
8	Actinidia deliciosa	Kiwi	Unassigned	Unassigned	Actinidia Act d 11 Kirola MLP	150	332319679	12
9	Actinidia chinensis	Kiwi	Unassigned	Food Plant	Actinidia Kiwellin	189	85701136	7
10	Actinidia deliciosa	Kiwi	Unassigned	Food Plant	Actinidia Phytocystatin Act d 14	116	40807635	7
11	Actinidia chinensis	Kiwi	Unassigned	Food Plant	Actinidia thaumatin Act d 2	20	68064399	7
12	Actinidia deliciosa	Kiwi	Act c 2	Food Plant	Actinidia thaumatin Act d 2	225	71057064	7
13	Actinidia deliciosa	Kiwi	Unassigned	Food Plant	Actinidia thaumatin Act d 2	201	146737976	9
14	Aedes aegypti	Yellow fever mosquito	Aed a 2	Venom or Salivary	Aedes Aed a 2	321	118216	7
15	Aedes aegypti	Yellow fever mosquito	Aed a 2	Venom or Salivary	Aedes Aed a 2	321	205525919	9
16	Aedes aegypti	Yellow fever mosquito	Unassigned	Venom or Salivary	Aedes Aed a 3	253	2114497	7
17	Aedes aegypti	Yellow fever mosquito	Unassigned	Venom or Salivary	Aedes Aed a 3	273	94468546	7
18	Aedes aegypti	Yellow fever mosquito	Unassigned	Venom or Salivary	Aedes Aed a 3	258	94468552	7
19	Aedes aegypti	Yellow fever mosquito	Aed a 1	Venom or Salivary	Aedes apyrase Aed a	562	556272	7

		mosquito		Salivary	1			
20	Aedes aegypti	Yellow fever mosquito	Unassigned	Venom or Salivary	Aedes apyrase Aed a 1	562	193806340	10
21	Agrostis alba	Bent grass	Unassigned	Aero Plant	Agrostis Agr a 1	26	320606	7
22	Agrostis alba	Bent grass	Unassigned	Aero Plant	Agrostis Agr a 1	35	75139987	7
23	Agrostis alba	Bent grass	Unassigned	Aero Plant	Agrostis Agr a 1	35	75139989	7
24	Alnus glutinosa	Alder	Aln g 1	Aero Plant	Alnus Aln g 1	160	261407	7
25	Alnus glutinosa	Alder	Unassigned	Aero Plant	Alnus Aln g 4	85	3319651	7
26	Alternaria alternata	Fungus	Unassigned	Aero Fungi	Alternaria ADH Alta a 10	497	76666767	7
27	Alternaria alternata	Fungus	Unassigned	Aero Fungi	Alternaria Alt a 13	231	74611808	10
28	Alternaria alternata	Fungus	Alt a 1	Aero Fungi	Alternaria Alt a I	157	1842045	7
29	Alternaria alternata	Fungus	Alt a 1	Aero Fungi	Alternaria Alt a I	115	21913174	7
30	Alternaria alternata	Fungus	Unassigned	Aero Fungi	Alternaria Alt a I	157	45680856	7
31	Alternaria alternata	Fungus	Alt a 6	Aero Fungi	Alternaria enolase Alt a 6	438	14423684	7
32	Alternaria alternata	Fungus	Unassigned	Aero Fungi	Alternaria flavodoxin Alt a 7	204	1168402	9
33	Alternaria alternata	Fungus	Alt a 3	Aero Fungi	Alternaria HSP Alt a 3	152	14423730	7
34	Alternaria alternata	Fungus	Unassigned	Aero Plant	Alternaria MnSOD	25	292630881	12
35	Alternaria alternata	Fungus	Unassigned	Aero Fungi	Alternaria Nuc Transport 2	124	21748153	7
36	Alternaria alternata	Fungus	Alt a 12	Aero Fungi	Alternaria Ribosomal BP P1 Alt a 12	110	1350779	7
37	Alternaria alternata	Fungus	Alt a 5	Aero Fungi	Alternaria ribosomal P2 Alt a 5	113	1850540	7
38	Alternaria alternata	Fungus	Unassigned	Aero Fungi	Alternaria ribosomal P2 Alt a 5	113	1173071	10
39	Alternaria alternata	Fungus	Unassigned	Aero Fungi	Alternaria TCTP IgE binding	169	112824341	11
40	Alternaria alternata	Fungus	Alt a 4	Aero Fungi	Alternaria thioredoxin Alt a 4	436	85701160	7
41	Amaranthus retroflexus	Common Amaranth	Unassigned	Aero Plant	Amaranthus Ama r 2 Proflin	133	227937304	10
42	Ambrosia artemisiifolia	Short ragweed	Amb a 1.1	Aero Plant	Ambrosia Amb a 1	396	113475	7

43	Ambrosia  artemisiifolia	Short ragweed	Amb a 1.2	Aero Plant	Ambrosia Amb a 1	398	113476	7
44	Ambrosia  artemisiifolia	Short ragweed	Amb a 1.3	Aero Plant	Ambrosia Amb a 1	397	113477	7
45	Ambrosia  artemisiifolia	Short ragweed	Amb a 1.4	Aero Plant	Ambrosia Amb a 1	392	113478	7
46	Ambrosia  artemisiifolia	Short ragweed	Amb a 1.3	Aero Plant	Ambrosia Amb a 1	397	166443	7
47	Ambrosia  artemisiifolia	Short ragweed	Unassigned	Aero Plant	Ambrosia Amb a 1	396	302127810	12
48	Ambrosia  artemisiifolia	Short ragweed	Unassigned	Aero Plant	Ambrosia Amb a 1	398	302127812	12
49	Ambrosia  artemisiifolia	Short ragweed	Unassigned	Aero Plant	Ambrosia Amb a 1	397	302127814	12
50	Ambrosia  artemisiifolia	Short ragweed	Unassigned	Aero Plant	Ambrosia Amb a 1	397	302127816	12
51	Ambrosia  artemisiifolia	Short ragweed	Unassigned	Aero Plant	Ambrosia Amb a 1	397	302127818	12
52	Ambrosia  artemisiifolia	Short ragweed	Unassigned	Aero Plant	Ambrosia Amb a 1	397	302127820	12
53	Ambrosia  artemisiifolia	Short ragweed	Unassigned	Aero Plant	Ambrosia Amb a 1	397	302127822	12
54	Ambrosia  artemisiifolia	Short ragweed	Unassigned	Aero Plant	Ambrosia Amb a 1	387	302127824	12
55	Ambrosia  artemisiifolia	Short ragweed	Unassigned	Aero Plant	Ambrosia Amb a 1	397	302127826	12
56	Ambrosia  artemisiifolia	Short ragweed	Unassigned	Aero Plant	Ambrosia Amb a 1	397	302127828	12
57	Ambrosia  artemisiifolia	Short ragweed	Amb a 2	Aero Plant	Ambrosia Amb a 2	397	113479	7
58	Ambrosia  artemisiifolia  (elatiior)	Short ragweed	Amb a 3	Aero Plant	Ambrosia Amb a 3	101	416636	7
59	Ambrosia  artemisiifolia	Short ragweed	Unassigned	Aero Plant	Ambrosia Amb a 4	164	285005079	11
60	Ambrosia  artemisiifolia	Short ragweed	Unassigned	Aero Plant	Ambrosia Amb a 4	164	291197394	12
61	Ambrosia  artemisiifolia	Short ragweed	Unassigned	Aero Plant	Ambrosia Amb a 4	111	291482306	12
62	Ambrosia  artemisiifolia	Short ragweed	Unassigned	Aero Plant	Ambrosia Amb a 4	140	291482308	12
63	Ambrosia  artemisiifolia	Short ragweed	Unassigned	Aero Plant	Ambrosia Amb a 4	134	291482310	12
64	Ambrosia  artemisiifolia	Short ragweed	Unassigned	Aero Plant	Ambrosia Amb a 4	96	291482314	12
65	Ambrosia  artemisiifolia	Short ragweed	Unassigned	Aero Plant	Ambrosia Amb a 4	110	291482316	12

66	Ambrosia artemisiifolia	Short ragweed	Unassigned	Aero Plant	Ambrosia Amb a 4	116	291482318	12
67	Ambrosia artemisiifolia (elator)	Short ragweed	Amb a 5	Aero Plant	Ambrosia Amb a 5	45	114090	7
68	Ambrosia psilostachya	Western ragweed	Unassigned	Aero Plant	Ambrosia Amb a 5	77	515953	7
69	Ambrosia psilostachya	Western ragweed	Unassigned	Aero Plant	Ambrosia Amb a 5	77	515954	7
70	Ambrosia psilostachya	Western ragweed	Unassigned	Aero Plant	Ambrosia Amb a 5	77	515955	7
71	Ambrosia psilostachya	Western ragweed	Unassigned	Aero Plant	Ambrosia Amb a 5	77	515956	7
72	Ambrosia psilostachya	Western ragweed	Unassigned	Aero Plant	Ambrosia Amb a 5	77	515957	7
73	Ambrosia artemisiifolia	Short ragweed	Amb a 6	Aero Plant	Ambrosia Amb a 6	118	14285595	7
74	Ambrosia artemisiifolia	Short ragweed	Unassigned	Aero Plant	Ambrosia Amb a 8 profilin	133	34851182	7
75	Ambrosia artemisiifolia	Short ragweed	Unassigned	Aero Plant	Ambrosia Amb a 8 profilin	131	34851180	7
76	Ambrosia artemisiifolia	Short ragweed	Unassigned	Aero Plant	Ambrosia Amb a 8 profilin	131	34851178	7
77	Ambrosia artemisiifolia	Short ragweed	Unassigned	Aero Plant	Ambrosia Amb a 8 profilin	133	62249502	7
78	Ambrosia artemisiifolia	Short ragweed	Unassigned	Aero Plant	Ambrosia Amb a 8 profilin	133	62249512	7
79	Ambrosia trifida	Giant ragweed	Amb t 5	Aero Plant	Ambrosia trifida Amb t 5	73	114091	7
80	Anacardium occidentale	Cashew	Ana o 1	Food Plant	Anacardium Ana o 1	536	21666498	7
81	Anacardium occidentale	Cashew	Ana o 1	Food Plant	Anacardium Ana o 1	538	21914823	7
82	Anacardium occidentale	Cashew	Ana o 2	Food Plant	Anacardium Ana o 2	457	25991543	7
83	Anacardium occidentale	Cashew	Ana o 3	Food Plant	Anacardium Ana o 3	138	24473800	7
84	Ananas comosus	Pineapple	Unassigned	Aero Plant	Ananas Ana c 2 Bromelain precursor	351	75277440	7
85	Ananas comosus	Pineapple	Unassigned	Food Plant	Ananas profilin	131	75306610	10
86	Anisakis simplex	Parasitic fish worm	Ani s 1	Worm (parasite)	Anisakis Ani s 1 protease inhibitor	194	47605452	7
87	Anisakis simplex	Parasitic fish worm	Unassigned	Food Animal	Anisakis Ani s 11	307	323575361	12
88	Anisakis simplex	Parasitic fish worm	Unassigned	Food Animal	Anisakis Ani s 11	160	323575363	12

89	Anisakis simplex	Parasitic fish worm	Unassigned	Food Animal	Anisakis Ani s 11	287	323575365	12
90	Anisakis simplex	Parasitic fish worm	Unassigned	Food Animal	Anisakis Ani s 12	295	323575367	12
91	Anisakis simplex	Parasitic fish worm	Ani s 2	Worm (parasite)	Anisakis Ani s 2 paramyosin	473	8453086	7
92	Anisakis simplex	Parasitic fish worm	Unassigned	Worm (parasite)	Anisakis Ani s 2 paramyosin	869	42559536	9
93	Anisakis simplex	Parasitic fish worm	Ani s 4	Worm (parasite)	Anisakis Ani s 4	14	47605398	7
94	Anisakis simplex	Parasitic fish worm	Unassigned	Worm (parasite)	Anisakis Ani s 4	115	110346534	8
95	Anisakis simplex	Parasitic fish worm	Unassigned	Worm (parasite)	Anisakis Ani s 5 SXP/RAL-2 family protein	152	121308878	8
96	Anisakis simplex	Parasitic fish worm	Unassigned	Worm (parasite)	Anisakis Ani s 7 UA3-recognized allergen	1096	119524036	9
97	Anisakis simplex	Parasitic fish worm	Unassigned	Worm (parasite)	Anisakis Ani s 8 SXP/RAL-2 family protein 2	150	155676636	9
98	Anisakis simplex	Parasitic fish worm	Unassigned	Worm (parasite)	Anisakis Ani s 8 SXP/RAL-2 family protein 2	150	155676682	9
99	Anisakis simplex	Parasitic fish worm	Unassigned	Worm (parasite)	Anisakis Ani s 8 SXP/RAL-2 family protein 2	150	155676684	9
100	Anisakis simplex	Parasitic fish worm	Unassigned	Worm (parasite)	Anisakis Ani s 8 SXP/RAL-2 family protein 2	150	155676686	9
101	Anisakis simplex	Parasitic fish worm	Unassigned	Worm (parasite)	Anisakis Ani s 8 SXP/RAL-2 family protein 2	150	155676688	9
102	Anisakis simplex	Parasitic fish worm	Unassigned	Worm (parasite)	Anisakis Ani s 8 SXP/RAL-2 family protein 2	150	155676690	9
103	Anisakis simplex	Parasitic fish worm	Unassigned	Worm (parasite)	Anisakis Ani s 8 SXP/RAL-2 family protein 2	150	155676692	9
104	Anisakis simplex	Parasitic fish worm	Unassigned	Worm (parasite)	Anisakis Ani s 8 SXP/RAL-2 family protein 2	150	155676694	9
105	Anisakis simplex	Parasitic fish worm	Unassigned	Worm (parasite)	Anisakis Ani s 8 SXP/RAL-2 family protein 2	150	155676696	9
106	Anisakis simplex	Parasitic fish worm	Unassigned	Worm (parasite)	Anisakis Ani s 8 SXP/RAL-2 family protein 2	150	155676698	9
107	Anisakis simplex	Parasitic fish worm	Unassigned	Worm (parasite)	Anisakis Ani s 9	147	157418806	9

108	Anisakis simplex	Parasitic fish worm	Unassigned	Worm (parasite)	Anisakis simplex troponin-like	161	6065738	7
109	Anthoxanthum odoratum	Sweet vernal grass	Unassigned	Aero Plant	Anthoxanthum Ant o 1	26	320607	7
110	Anthoxanthum odoratum	Sweet vernal grass	Unassigned	Aero Plant	Anthoxanthum Ant o 1	32	75139986	7
111	Anthoxanthum odoratum	Sweet vernal grass	Unassigned	Aero Plant	Anthoxanthum Ant o 1	32	75139990	7
112	Apis cerana	Indian honeybee	Unassigned	Venom or Salivary	Apis Api m 1	134	7435005	7
113	Apis cerana cerana	Indian honeybee	Unassigned	Venom or Salivary	Apis Api m 1	134	24638082	7
114	Apis dorsata	Giant honeybee	Unassigned	Venom or Salivary	Apis Api m 1	134	47117012	7
115	Apis mellifera	Honeybee	Api m 1	Venom or Salivary	Apis Api m 1	167	24418862	7
116	Apis mellifera	Honeybee	Api m 2	Venom or Salivary	Apis Api m 2	382	585279	7
117	Apis mellifera	Honeybee	Unassigned	Venom or Salivary	Apis Api m 3 acid phosphatase	388	208342441	10
118	Apis mellifera	Honeybee	Unassigned	Venom or Salivary	Apis Api m 3 acid phosphatase	388	60652325	11
119	Apis dorsata	Giant honeybee	Unassigned	Venom or Salivary	Apis Api m 4 Melittin	26	126955	7
120	Apis mellifera	Honeybee	Unassigned	Venom or Salivary	Apis Api m 4 Melittin	27	69552	7
121	Apis mellifera	Honeybee	Unassigned	Venom or Salivary	Apis Api m 4 Melittin	70	126949	8
122	Apis mellifera	Honeybee	Unassigned	Venom or Salivary	Apis Api m 5 dipeptidylpeptidase	775	313471719	12
123	Apis mellifera	Honeybee	Unassigned	Venom or Salivary	Apis Api m 6	92	94400907	7
124	Apis mellifera	Honeybee	Unassigned	Venom or Salivary	Apis Api m 6	94	88770352	10
125	Apis mellifera	Honeybee	Unassigned	Venom or Salivary	Apis icarapin Api m 10	223	94471622	7
126	Apis mellifera	Honeybee	Unassigned	Venom or Salivary	Apis icarapin Api m 10	175	94471624	7
127	Apium graveolens	Celery	Api g 1.0101	Food Plant	Apium Api g 1	154	1346568	7
128	Apium graveolens	Celery	Api g 1.0201	Food Plant	Apium Api g 1	159	14423646	9
129	Apium graveolens	Celery	Api g 2.0101	Food Plant	Apium Api g 2	118	256600126	12
130	Apium graveolens	Celery	Api g 4	Food Plant	Apium Api g 4	134	4761578	7

131	Apium graveolens	Celery	Unassigned	Food Plant	Apium Api g 5	22	33300921	7
132	Apium graveolens	Celery	Unassigned	Food Plant	Apium Api g 5	30	32363124	7
133	Apium graveolens	Celery	Unassigned	Food Plant	Apium Api g 5	24	32363125	7
134	Apium graveolens	Celery	Unassigned	Food Plant	Apium Api g 5	10	32363126	7
135	Arachis hypogaea	Peanut	Unassigned	Food Plant	Arachis Agglutinin (lectin)	273	253289	7
136	Arachis hypogaea	Peanut	Ara h 1	Food Plant	Arachis Ara h 1	614	1168390	7
137	Arachis hypogaea	Peanut	Ara h 1	Food Plant	Arachis Ara h 1	626	1168391	7
138	Arachis hypogaea	Peanut	Unassigned	Food Plant	Arachis Ara h 1	299	46560474	7
139	Arachis hypogaea	Peanut	Unassigned	Food Plant	Arachis Ara h 1	303	46560472	7
140	Arachis hypogaea	Peanut	Unassigned	Food Plant	Arachis Ara h 1	428	46560476	7
141	Arachis hypogaea	Peanut	Unassigned	Food Plant	Arachis Ara h 1	619	312233063	12
142	Arachis hypogaea	Peanut	Ara h 2.02	Food Plant	Arachis Ara h 2	172	26245447	7
143	Arachis hypogaea	Peanut	Ara h 2	Food Plant	Arachis Ara h 2	169	31322017	7
144	Arachis hypogaea	Peanut	Unassigned	Food Plant	Arachis Ara h 2	156	15418705	10
145	Arachis hypogaea	Peanut	Unassigned	Food Plant	Arachis Ara h 2	158	224747150	10
146	Arachis hypogaea	Peanut	Ara h 5	Food Plant	Arachis Ara h 5	131	5902968	7
147	Arachis hypogaea	Peanut	Unassigned	Food Plant	Arachis Ara h 5	131	284810529	11
148	Arachis hypogaea	Peanut	Ara h 6	Food Plant	Arachis Ara h 6	129	5923742	7
149	Arachis hypogaea	Peanut	Unassigned	Food Plant	Arachis Ara h 6	144	17225991	7
150	Arachis hypogaea	Peanut	Unassigned	Food Plant	Arachis Ara h 6	127	159163254	9
151	Arachis hypogaea	Peanut	Unassigned	Food Plant	Arachis Ara h 6	145	75114094	10
152	Arachis hypogaea	Peanut	Ara h 7	Food Plant	Arachis Ara h 7	160	5931948	7
153	Arachis hypogaea	Peanut	Unassigned	Food Plant	Arachis Ara h 7	164	158121995	10
154	Arachis hypogaea	Peanut	Ara h 8	Food Plant	Arachis Ara h 8	157	37499626	7
155	Arachis hypogaea	Peanut	Unassigned	Food Plant	Arachis Ara h 8	153	145904610	9
156	Arachis hypogaea	Peanut	Unassigned	Food Plant	Arachis Ara h 8	157	169786740	9
157	Arachis hypogaea	Peanut	Unassigned	Food Plant	Arachis Ara h 8	157	110676574	12
158	Arachis hypogaea	Peanut	Unassigned	Food Plant	Arachis Ara h 9 LTP isoallergens	116	161087230	10
159	Arachis hypogaea	Peanut	Unassigned	Food Plant	Arachis Ara h 9 LTP isoallergens	92	161610580	10
160	Arachis hypogaea	Peanut	Ara h 3	Food Plant	Arachis Glycinin (Ara h 3/Ara h 4)	507	3703107	7
161	Arachis hypogaea	Peanut	Ara h 4	Food Plant	Arachis Glycinin (Ara h 3/Ara h 4)	530	5712199	7
162	Arachis hypogaea	Peanut	Unassigned	Food Plant	Arachis Glycinin (Ara h 3/Ara h 4)	538	21314465	7

163	Arachis hypogaea	Peanut	Unassigned	Food Plant	Arachis Glycinin (Ara h 3/Ara h 4)	219	22135348	7
164	Arachis hypogaea	Peanut	Unassigned	Food Plant	Arachis Glycinin (Ara h 3/Ara h 4)	512	112380623	8
165	Arachis hypogaea	Peanut	Unassigned	Food Plant	Arachis Glycinin (Ara h 3/Ara h 4)	530	199732457	10
166	Arachis hypogaea	Peanut	Unassigned	Food Plant	Arachis Glycinin (Ara h 3/Ara h 4)	510	224036293	10
167	Arachis hypogaea	Peanut	Unassigned	Food Plant	Arachis Glycinin (Ara h 3/Ara h 4)	512	312233065	12
168	Argas reflexus	European pigeon tick	Arg r 1	Venom or Salivary	Argas Arg r 1	159	58371884	7
169	Argas reflexus	European pigeon tick	Unassigned	Venom or Salivary	Argas Arg r 1	144	322812205	12
170	Artemisia vulgaris	Mugwort	Unassigned	Aero Plant	Artemisia Amb a 1-like	396	62530263	8
171	Artemisia vulgaris	Mugwort	Art v 1	Aero Plant	Artemisia Art v 1	132	27818335	7
172	Artemisia vulgaris	Mugwort	Unassigned	Aero Plant	Artemisia Art v 2	162	148887203	9
173	Artemisia vulgaris	Mugwort	Unassigned	Aero Plant	Artemisia Art v 3	37	73621307	7
174	Artemisia vulgaris	Mugwort	Unassigned	Aero Plant	Artemisia Art v 3	114	189544578	11
175	Artemisia vulgaris	Mugwort	Unassigned	Aero Plant	Artemisia Art v 3	116	189544584	11
176	Artemisia vulgaris	Mugwort	Unassigned	Aero Plant	Artemisia Art v 3	117	189544590	11
177	Artemisia vulgaris	Mugwort	Unassigned	Aero Plant	Artemisia Art v 3	117	189544595	11
178	Artemisia vulgaris	Mugwort	Unassigned	Aero Plant	Artemisia Art v 4	133	73621415	7
179	Artemisia vulgaris	Mugwort	Unassigned	Aero Plant	Artemisia Art v 4	133	73621416	7
180	Ascaris lumbricoides	Parasitic roundworm	Unassigned	Worm (parasite)	Ascaris Asc s 1	134	2735096	7
181	Ascaris lumbricoides	Parasitic roundworm	Unassigned	Worm (parasite)	Ascaris Asc s 1	134	2735098	7
182	Ascaris lumbricoides	Parasitic roundworm	Unassigned	Worm (parasite)	Ascaris Asc s 1	133	2735102	7
183	Ascaris lumbricoides	Parasitic roundworm	Unassigned	Worm (parasite)	Ascaris Asc s 1	133	2735106	7
184	Ascaris lumbricoides	Parasitic roundworm	Unassigned	Worm (parasite)	Ascaris Asc s 1	267	2735108	7
185	Ascaris lumbricoides	Parasitic roundworm	Unassigned	Worm (parasite)	Ascaris Asc s 1	267	2735110	7

186	Ascaris lumbricoides	Parasitic roundworm	Unassigned	Worm (parasite)	Ascaris Asc s 1	267	2735112	7
187	Ascaris lumbricoides	Parasitic roundworm	Unassigned	Worm (parasite)	Ascaris Asc s 1	134	2735114	7
188	Ascaris lumbricoides	Parasitic roundworm	Unassigned	Worm (parasite)	Ascaris Asc s 1	134	2735118	7
189	Ascaris lumbricoides	Parasitic roundworm	Unassigned	Worm (parasite)	Ascaris Asc s 1	134	2735100	7
190	Ascaris lumbricoides	Parasitic roundworm	Unassigned	Worm (parasite)	Ascaris Asc s 1	133	2735104	11
191	Ascaris suum	Parasitic roundworm	Asc s 1	Worm (parasite)	Ascaris Asc s 1	68	299550	7
192	Ascaris suum	Parasitic roundworm	Asc s 1	Worm (parasite)	Ascaris Asc s 1	1365	77416849	7
193	Ascaris suum	Parasitic roundworm	Unassigned	Worm (parasite)	Ascaris Asc s 1	134	343197079	12
194	Ascaris lumbricoides	Parasitic roundworm	Unassigned	Worm (parasite)	Ascaris tropomyosin	287	224016002	10
195	Aspergillus oryzae	Fungus	Asp o 21	Aero Fungi	Aspergillus Alpha-amylase A	499	94706935	7
196	Aspergillus fumigatus	Fungus	Asp f 1	Aero Fungi	Aspergillus Asp f 1	125	3021324	7
197	Aspergillus fumigatus	Fungus	Asp f 1	Aero Fungi	Aspergillus Asp f 1	150	9280360	7
198	Aspergillus fumigatus	Fungus	Unassigned	Aero Fungi	Aspergillus Asp f 1	176	54039254	7
199	Aspergillus fumigatus	Fungus	Asp f 10	Aero Fungi	Aspergillus Asp f 10	395	963013	7
200	Aspergillus fumigatus	Fungus	Asp f 11	Aero Fungi	Aspergillus Asp f 11	178	5019414	7
201	Aspergillus fumigatus	Fungus	Asp f 12	Aero Fungi	Aspergillus Asp f 12	706	83303658	7
202	Aspergillus fumigatus	Fungus	Asp f 2	Aero Fungi	Aspergillus Asp f 2	250	664852	7
203	Aspergillus fumigatus	Fungus	Asp f 2	Aero Fungi	Aspergillus Asp f 2	310	83300352	7
204	Aspergillus fumigatus Af293	Fungus	Unassigned	Aero Fungi	Aspergillus Asp f 2	304	66849502	7
205	Aspergillus fumigatus	Fungus	Asp f 22	Aero Fungi	Aspergillus Asp f 22	438	13925873	7
206	Aspergillus fumigatus	Fungus	Unassigned	Aero Fungi	Aspergillus Asp f 22	438	83288046	7
207	Aspergillus fumigatus	Fungus	Asp f 3	Aero Fungi	Aspergillus Asp f 3	168	2769700	7
208	Aspergillus fumigatus Af293	Fungus	Unassigned	Aero Fungi	Aspergillus Asp f 3	168	66845476	8

209	Aspergillus fumigatus	Fungus	Asp f 4	Aero Fungi	Aspergillus Asp f 4	286	3005839	17
210	Aspergillus fumigatus	Fungus	Unassigned	Aero Fungi	Aspergillus Asp f 4	322	83300369	17
211	Aspergillus fumigatus Af293	Fungus	Unassigned	Aero Fungi	Aspergillus Asp f 4	322	66847146	18
212	Aspergillus fumigatus	Fungus	Asp f 6	Aero Fungi	Aspergillus Asp f 6	221	1648970	17
213	Aspergillus fumigatus	Fungus	Unassigned	Aero Fungi	Aspergillus Asp f 6	210	83305645	17
214	Aspergillus fumigatus	Fungus	Asp f 7	Aero Fungi	Aspergillus Asp f 7	270	83300389	17
215	Aspergillus fumigatus	Fungus	Asp f 8	Aero Fungi	Aspergillus Asp f 8	111	6686524	17
216	Aspergillus fumigatus	Fungus	Unassigned	Aero Fungi	Aspergillus Asp f 8	111	83305635	17
217	Aspergillus fumigatus	Fungus	Unassigned	Aero Fungi	Aspergillus Asp f 9	395	85540942	17
218	Aspergillus niger	Fungus	Asp n 14	Aero Fungi	Aspergillus Asp n 14	804	2181180	17
219	Aspergillus niger	Fungus	Asp n 14	Aero Fungi	Aspergillus Asp n 14	804	4235093	17
220	Aspergillus flavus	Fungus	Unassigned	Aero Fungi	Aspergillus Oryzin Asp o 13, fl 13	403	74665726	17
221	Aspergillus oryzae	Fungus	Asp o 13	Aero Fungi	Aspergillus Oryzin Asp o 13, fl 13	403	129235	17
222	Aspergillus fumigatus	Fungus	Unassigned	Aero Fungi	Aspergillus Ribosomal protein L3	392	21215170	17
223	Aspergillus fumigatus	Fungus	Unassigned	Aero Fungi	Aspergillus Ribosomal protein L3	392	83305621	17
224	Aspergillus fumigatus	Fungus	Unassigned	Aero Fungi	Aspergillus Vacuolar Serine protease	495	2143220	17
225	Aspergillus niger	Fungus	Unassigned	Aero Fungi	Aspergillus Vacuolar Serine protease	533	289172	17
226	Bacillus sp.		Unassigned	Bacteria airway	Bacillus lentus Esperase	361	1225905	19
227	Bacillus lentus		Unassigned	Bacteria airway	Bacillus lentus subtilisin	269	267048	19
228	Bacillus licheniformis		Unassigned	Bacteria airway	Bacillus licheniformis subtilisin	379	135016	19
229	Bacillus licheniformis		Unassigned	Bacteria airway	Bacillus licheniformis subtilisin	374	11127680	19

230	Balanus rostratus		Unassigned	Food Animal	Balanus r tropomyosin	284	125659386	19
231	Batillus cornutus	Japanese turban shell	Unassigned	Food Animal	Batillus Tur c1	20	47117350	17
232	Batillus cornutus	Japanese turban shell	Unassigned	Food Animal	Batillus Tur c1	27	47117351	17
233	Batillus cornutus	Japanese turban shell	Unassigned	Food Animal	Batillus Tur c1	284	219806588	10
234	Bertholletia excelsa	Brazil nut	Ber e 2	Food Plant	Bertholletia 11S globulin	465	30313867	17
235	Bertholletia excelsa	Brazil nut	Unassigned	Food Plant	Bertholletia Ber e 1	154	17713	17
236	Bertholletia excelsa	Brazil nut	Ber e 1	Food Plant	Bertholletia Ber e 1	146	112754	17
237	Betula pendula white birch	European white birch	Bet v 2	Aero Plant	Bet v 2	133	130975	17
238	Betula pendula white birch	European white birch	Unassigned	Aero Plant	Bet v 2	133	157830684	19
239	Betula pendula white birch	European white birch	Bet v 1	Aero Plant	Betula Bet v 1	51	320545	17
240	Betula pendula white birch	European white birch	Bet v 1	Aero Plant	Betula Bet v 1	160	534898	17
241	Betula pendula white birch	European white birch	Bet v 1	Aero Plant	Betula Bet v 1	159	534900	17
242	Betula pendula white birch	European white birch	Bet v 1	Aero Plant	Betula Bet v 1	160	534910	17
243	Betula pendula white birch	European white birch	Bet v 1.0301	Aero Plant	Betula Bet v 1	160	1168702	17
244	Betula pendula white birch	European white birch	Bet v 1.1001	Aero Plant	Betula Bet v 1	160	1168709	17
245	Betula pendula white birch	European white birch	Bet v 1.1601	Aero Plant	Betula Bet v 1	160	1321714	17
246	Betula pendula white birch	European white birch	Bet v 1.1701	Aero Plant	Betula Bet v 1	160	1321716	17
247	Betula pendula white birch	European white birch	Bet v 1.1801	Aero Plant	Betula Bet v 1	160	1321718	17
248	Betula pendula white birch	European white birch	Bet v 1.1502	Aero Plant	Betula Bet v 1	160	1321720	17
249	Betula pendula white birch	European white birch	Bet v 1.1901	Aero Plant	Betula Bet v 1	160	1321722	17
250	Betula pendula white birch	European white birch	Bet v 1.2001	Aero Plant	Betula Bet v 1	160	1321724	17
251	Betula pendula white birch	European white birch	Bet v 1.2101	Aero Plant	Betula Bet v 1	160	1321726	17
252	Betula pendula white birch	European white birch	Bet v 1.2201	Aero Plant	Betula Bet v 1	160	1321728	17

253	Betula pendula white birch	European	Bet v 1	Aero Plant	Betula Bet v 1	160	1168703	17
254	Betula pendula white birch	European	Bet v 1.0501	Aero Plant	Betula Bet v 1	160	1168704	17
255	Betula pendula white birch	European	Bet v 1f/I	Aero Plant	Betula Bet v 1	160	1168705	17
256	Betula pendula white birch	European	Bet v 1.0801	Aero Plant	Betula Bet v 1	160	1168707	17
257	Betula pendula white birch	European	Bet v 1.0901	Aero Plant	Betula Bet v 1	160	1168708	17
258	Betula pendula white birch	European	Bet v 1m/n	Aero Plant	Betula Bet v 1	160	1168710	17
259	Betula pendula white birch	European	Bet v 1.0201	Aero Plant	Betula Bet v 1	160	1168701	17
260	Betula pendula white birch	European	Bet v 1.2401	Aero Plant	Betula Bet v 1	160	1542861	17
261	Betula pendula white birch	European	Bet v 1.2501	Aero Plant	Betula Bet v 1	160	1542863	17
262	Betula pendula white birch	European	Bet v 1.2601	Aero Plant	Betula Bet v 1	160	1542865	17
263	Betula pendula white birch	European	Bet v 1.2701	Aero Plant	Betula Bet v 1	160	1542867	17
264	Betula pendula white birch	European	Bet v 1.2801	Aero Plant	Betula Bet v 1	160	1542869	17
265	Betula pendula white birch	European	Bet v 1.2901	Aero Plant	Betula Bet v 1	160	1542871	17
266	Betula pendula white birch	European	Bet v 1.3001	Aero Plant	Betula Bet v 1	160	1542873	17
267	Betula pendula white birch	European	Bet v 1.2301	Aero Plant	Betula Bet v 1	160	2414158	17
268	Betula pendula white birch	European	Bet v 1	Aero Plant	Betula Bet v 1	160	2564220	17
269	Betula pendula white birch	European	Bet v 1	Aero Plant	Betula Bet v 1	160	2564222	17
270	Betula pendula white birch	European	Bet v 1	Aero Plant	Betula Bet v 1	160	2564224	17
271	Betula pendula white birch	European	Bet v 1	Aero Plant	Betula Bet v 1	160	2564228	17
272	Betula pendula white birch	European	Bet v 1	Aero Plant	Betula Bet v 1	160	4006928	17
273	Betula pendula white birch	European	Bet v 1	Aero Plant	Betula Bet v 1	160	4006945	17
274	Betula pendula white birch	European	Bet v 1	Aero Plant	Betula Bet v 1	160	4006953	17
275	Betula pendula white birch	European	Bet v 1	Aero Plant	Betula Bet v 1	160	4006955	17

276	Betula pendula white birch	European	Bet v 1	Aero Plant	Betula Bet v 1	160	4006957	17
277	Betula pendula white birch	European	Bet v 1	Aero Plant	Betula Bet v 1	160	4006959	17
278	Betula pendula white birch	European	Bet v 1	Aero Plant	Betula Bet v 1	160	4006961	17
279	Betula pendula white birch	European	Bet v 1	Aero Plant	Betula Bet v 1	160	4006965	17
280	Betula pendula white birch	European	Bet v 1	Aero Plant	Betula Bet v 1	160	4006967	17
281	Betula pendula white birch	European	Bet v 1	Aero Plant	Betula Bet v 1	159	4376216	17
282	Betula pendula white birch	European	Bet v 1	Aero Plant	Betula Bet v 1	159	4376219	17
283	Betula pendula white birch	European	Bet v 1	Aero Plant	Betula Bet v 1	159	4376220	17
284	Betula pendula white birch	European	Bet v 1	Aero Plant	Betula Bet v 1	159	4376221	17
285	Betula pendula white birch	European	Bet v 1	Aero Plant	Betula Bet v 1	159	4376222	17
286	Betula pendula white birch	European	Bet v 1 b1	Aero Plant	Betula Bet v 1	160	4590392	17
287	Betula pendula white birch	European	Bet v 1 b2	Aero Plant	Betula Bet v 1	160	4590394	17
288	Betula pendula white birch	European	Bet v 1 b3	Aero Plant	Betula Bet v 1	160	4590396	17
289	Betula pendula white birch	European	Bet v 1.0701	Aero Plant	Betula Bet v 1	160	1168706	17
290	Betula pendula white birch	European	Bet v 1	Aero Plant	Betula Bet v 1	159	11514622	17
291	Betula pendula white birch	European	Bet v 1x	Aero Plant	Betula Bet v 1	21	30908931	17
292	Betula pendula white birch	European	Bet v 1	Aero Plant	Betula Bet v 1	159	38492423	17
293	Betula pendula white birch	European	Unassigned	Aero Plant	Betula Bet v 1	43	239734	17
294	Betula pendula white birch	European	Unassigned	Aero Plant	Betula Bet v 1	120	4006963	17
295	Betula pendula white birch	European	Unassigned	Aero Plant	Betula Bet v 1	120	4006947	17
296	Betula pendula white birch	European	Bet v 1	Aero Plant	Betula Bet v 1	160	114922	18
297	Betula pendula white birch	European	Bet v 1	Aero Plant	Betula Bet v 1	159	159162097	19
298	Betula platyphylla	Japanese	Unassigned	Aero Plant	Betula Bet v 1	160	12583681	17

299	Betula  platyphylla	Japanese  white birch	Unassigned	Aero Plant	Betula Bet v 1	160	12583683	7
300	Betula  platyphylla	Japanese  white birch	Unassigned	Aero Plant	Betula Bet v 1	160	12583685	7
301	Betula sp.	Birch	Unassigned	Aero Plant	Betula Bet v 1	51	298736	7
302	Betula pendula	European  white birch	Unassigned	Aero Plant	Betula Bet v 1b	51	320546	7
303	Betula sp.	Birch	Unassigned	Aero Plant	Betula Bet v 1b	51	298737	7
304	Betula pendula	European  white birch	Bet v 3	Aero Plant	Betula Bet v 3	205	1168696	7
305	Betula pendula	European  white birch	Bet v 4	Aero Plant	Betula Bet v 4	85	14423850	7
306	Betula pendula	European  white birch	Bet v  6.0102	Aero Plant	Betula Bet v 6	308	10764491	7
307	Betula pendula	European  white birch	Bet v 7	Aero Plant	Betula Bet v 7	173	21886603	7
308	Blattella  germanica	German  cockroach	Bla g 1.02	Aero  Insect	Blattella Bla g 1	492	4240395	7
309	Blattella  germanica	German  cockroach	Bla g  1.0101	Aero  Insect	Blattella Bla g 1	412	4572592	7
310	Blattella  germanica	German  cockroach	Bla g 2	Aero  Insect	Blattella Bla g 2	352	1703445	7
311	Blattella  germanica	German  cockroach	Unassigned	Aero  Insect	Blattella Bla g 2	330	62738637	7
312	Blattella  germanica	German  cockroach	Unassigned	Aero  Insect	Blattella Bla g 2	352	145105726	9
313	Blattella  germanica	German  cockroach	Unassigned	Aero  Insect	Blattella Bla g 2	334	315113421	12
314	Blattella  germanica	German  cockroach	Bla g 4	Aero  Insect	Blattella Bla g 4	182	1166573	7
315	Blattella  germanica	German  cockroach	Unassigned	Aero  Insect	Blattella Bla g 4	182	144952778	9
316	Blattella  germanica	German  cockroach	Unassigned	Aero  Insect	Blattella Bla g 4	181	212675308	10
317	Blattella  germanica	German  cockroach	Unassigned	Aero  Insect	Blattella Bla g 4	191	194350815	11
318	Blattella  germanica	German  cockroach	Unassigned	Aero  Insect	Blattella Bla g 4	190	194350817	11
319	Blattella  germanica	German  cockroach	Unassigned	Aero  Insect	Blattella Bla g 5	204	6225491	7
320	Blattella  germanica	German  cockroach	Unassigned	Aero  Insect	Blattella Bla g 5	200	144952780	9
321	Blattella  germanica	German  cockroach	Unassigned	Unassigned	Blattella Bla g 6	151	82704032	8
322	Blattella	German	Unassigned	Unassigned	Blattella Bla g 6	151	82704034	8

	germanica	cockroach						
323	Blattella germanica	German cockroach	Unassigned	Unassigned	Blattella Bla g 6	154	82704036	8
324	Blattella germanica	German cockroach	Unassigned	Aero Insect	Blattella delta GST	216	161137518	11
325	Blattella germanica	German cockroach	Unassigned	Aero Insect	Blattella tropomyosin	284	8101069	7
326	Blattella germanica	German cockroach	Unassigned	Aero Insect	Blattella uncertain	20	544618	7
327	Blattella germanica	German cockroach	Unassigned	Aero Insect	Blattella uncertain	25	544619	7
328	Blomia tropicalis	Mite	Blo t 1	Aero Mite	Blomia Blo t 1.01	221	14276828	7
329	Blomia tropicalis	Mite	Unassigned	Aero Mite	Blomia Blo t 1.02	333	33667928	8
330	Blomia tropicalis	Mite	Unassigned	Aero Mite	Blomia Blo t 1.02	333	2	8
331	Blomia tropicalis	Mite	Unassigned	Aero Mite	Blomia Blo t 10	284	156938889	9
332	Blomia tropicalis	Mite	Blo t 11	Aero Mite	Blomia Blo t 11	875	21954740	7
333	Blomia tropicalis	Mite	Unassigned	Aero Mite	Blomia Blo t 13.01	130	37958153	8
334	Blomia tropicalis	Mite	Unassigned	Aero Mite	Blomia Blo t 13.01	130	14423698	9
335	Blomia tropicalis	Mite	Unassigned	Aero Mite	Blomia Blo t 21 tentative	129	111120432	8
336	Blomia tropicalis	Mite	Unassigned	Aero Mite	Blomia Blo t 21 tentative	129	111494253	8
337	Blomia tropicalis	Mite	Unassigned	Aero Mite	Blomia Blo t 21 tentative	129	111120424	8
338	Blomia tropicalis	Mite	Unassigned	Aero Mite	Blomia Blo t 21 tentative	129	111120428	8
339	Blomia tropicalis	Mite	Unassigned	Aero Mite	Blomia Blo t 21 tentative	129	111120420	8
340	Blomia tropicalis	Mite	Unassigned	Aero Mite	Blomia Blo t 3	266	25989482	7
341	Blomia tropicalis	Mite	Unassigned	Aero Mite	Blomia Blo t 3	266	33667930	8
342	Blomia tropicalis	Mite	Blo t 5	Aero Mite	Blomia Blo t 5	134	4204917	7
343	Blomia tropicalis	Mite	Unassigned	Aero Mite	Blomia Blo t 5	134	111120436	9
344	Blomia tropicalis	Mite	Unassigned	Aero Mite	Blomia Blo t 5	134	111120450	9
345	Blomia	Mite	Unassigned	Aero Mite	Blomia Blo t 5	119	160285626	9

	tropicalis							
346	Bombus pennsylvanicus	Bumblebee	Unassigned	Venom or Salivary	Bombus Bom p 1 phospholipase	136	47117013	12
347	Bombus pennsylvanicus	Bumblebee	Unassigned	Venom or Salivary	Bombus Bom p 4 protease	243	75009997	12
348	Bombus terrestris	Bumblebee	Unassigned	Venom or Salivary	Bombus Bom t 1	136	14423832	7
349	Bombus terrestris	Bumblebee	Unassigned	Venom or Salivary	Bombus Bom t 4 protease	20	313471465	12
350	Bombyx mori	Silkworm	Unassigned	Food insect	Bombyx arginine kinase	355	204324083	10
351	Bos taurus	Bovine	Unassigned	Food Animal	Bos Alpha-s1 casein	93	162650	7
352	Bos taurus	Bovine	Unassigned	Food Animal	Bos Alpha-s1 casein	214	162792	7
353	Bos taurus	Bovine	Unassigned	Food Animal	Bos Alpha-s1 casein	214	162794	7
354	Bos taurus	Bovine	Unassigned	Food Animal	Bos Alpha-s1 casein	76	162927	7
355	Bos taurus	Bovine	Unassigned	Food Animal	Bos Alpha-s1 casein	205	159793197	9
356	Bos taurus	Bovine	Unassigned	Food Animal	Bos Alpha-s1 casein	172	159793201	9
357	Bos taurus	Bovine	Unassigned	Food Animal	Bos Alpha-s1 casein	129	159793217	9
358	Bos taurus	Bovine	Unassigned	Food Animal	Bos Alpha-s2-like casein	222	162929	7
359	Bos taurus	Bovine	Unassigned	Food Animal	Bos Beta-casein	224	162797	7
360	Bos taurus	Bovine	Unassigned	Food Animal	Bos Beta-casein	224	162805	7
361	Bos taurus	Bovine	Unassigned	Food Animal	Bos Beta-casein	224	162931	7
362	Bos taurus	Bovine	Unassigned	Food Animal	Bos Beta-casein	224	459292	7
363	Bos taurus	Bovine	Unassigned	Aero Animal	Bos Bos d 2	172	2497701	9
364	Bos taurus	Bovine	Bos d 3	Aero Animal	Bos Bos d 3	101	2493414	7
365	Bos taurus	Bovine	Bos d 4	Food Animal	Bos Bos d 4	142	295774	7
366	Bos taurus	Bovine	Unassigned	Food Animal	Bos Bos d 4	142	125996	9
367	Bos taurus	Bovine	Bos d 5	Food Animal	Bos Bos d 5	178	520	7
368	Bos taurus	Bovine	Unassigned	Food	Bos Bos d 5	14	162750	7

				Animal				
369	Bos taurus	Bovine	Unassigned	Food Animal	Bos Bos d 5	178	125910	9
370	Bos taurus	Bovine	Unassigned	Food Animal	Bos Bos d 5	178	195957138	10
371	Bos taurus	Bovine	Unassigned	Food Animal	Bos Bos d 6	607	3336842	7
372	Bos taurus	Bovine	Unassigned	Food Animal	Bos Bos d 6	607	1351907	10
373	Bos taurus	Bovine	Unassigned	Vaccine	Bos collagen alpha2	1364	27806257	11
374	Bos taurus	Bovine	Unassigned	Food Animal	Bos Kappa-casein	190	162811	7
375	Bos taurus	Bovine	Unassigned	Food Animal	Bos lactotransferrin	708	30794292	8
376	Brassica napus	Rape		Bra n 1 Food Plant	Bra n 1	125	75107016	9
377	Brassica juncea	Mustard		Bra j 1 Food Plant	Brassica Bra j 1 2S albumin	129	32363444	9
378	Brassica oleracea	Cabbage	Unassigned	Food Plant	Brassica Bra o 3 LTP manual entry	20	1	8
379	Brassica rapa subsp. rapa	Turnip	Unassigned	Contact	Brassica Bra r 2	91	32363456	9
380	Brassica napus	Rape	Unassigned	Aero Plant	Brassica Calcim binding protein Group I	79	59800143	7
381	Brassica rapa subsp. rapa	Turnip	Unassigned	Aero Plant	Brassica Calcim binding protein Group I	79	59800144	7
382	Brassica napus	Rape	Unassigned	Food Plant	Brassica napus 2S albumin	109	26985163	7
383	Brassica napus	Rape	Unassigned	Aero Plant	Brassica Polcalcic Group II	83	2129801	7
384	Brassica napus	Rape	Unassigned	Aero Plant	Brassica Polcalcic Group II	83	2129802	7
385	Brassica napus	Rape	Unassigned	Aero Plant	Brassica Polcalcic Group II	83	59800145	7
386	Brassica rapa	Turnip	Unassigned	Aero Plant	Brassica Polcalcic Group II	80	2129805	7
387	Brassica rapa subsp. rapa	Turnip	Unassigned	Aero Plant	Brassica Polcalcic Group II	83	59800146	7
388	Candida albicans	Yeast	Cand a 3	Contact	Candida Cand a 3	236	37548637	7
389	Candida albicans	Yeast	Unassigned	Contact	Candida Enolase 1	440	232054	7
390	Canis familiaris	Dog	Can f 1	Aero Animal	Canis Can f 1	174	3121745	7
391	Canis familiaris	Dog	Can f 2	Aero Animal	Canis Can f 2	180	3121746	7

392	Canis familiaris Dog	Can f 2	Aero	Canis Can f 2	177	29292272	7
			Animal				
393	Canis familiaris Dog	Can f 2	Aero	Canis Can f 2	179	29292274	7
			Animal				
394	Canis familiaris Dog	Can f 3	Aero	Canis Can f 3	265	633938	7
			Animal				
395	Canis familiaris Dog	Can f 3	Aero	Canis Can f 3	585	3319897	7
			Animal				
396	Canis familiaris Dog	Can f 3	Aero	Canis Can f 3	608	6687188	7
			Animal				
397	Canis familiaris Dog	Unassigned	Aero	Canis Can f	174	262232390	12
			Animal	epithelial 18 kDa			
398	Capsicum annuum  Bell pepper	Cap a 2	Food Plant	Capsicum Cap a 2	131	16555785	7
399	Carica papaya  Papaya	Unassigned	Food Plant	Carica Car p 1	345	129614	9
400	Carpinus betulus Hornbeam	Car b 1	Aero Plant	Carpinus Car b 1	159	402745	7
401	Carpinus betulus Hornbeam	Car b 1	Aero Plant	Carpinus Car b 1	160	730048	7
402	Carpinus betulus Hornbeam	Car b 1	Aero Plant	Carpinus Car b 1	160	730049	7
403	Carpinus betulus Hornbeam	Car b	Aero Plant	Carpinus Car b 1	160	1545875	7
		1.0103					
404	Carpinus betulus Hornbeam	Car b	Aero Plant	Carpinus Car b 1	160	1545877	7
		1.0104					
405	Carpinus betulus Hornbeam	Car b	Aero Plant	Carpinus Car b 1	160	1545879	7
		1.0104					
406	Carpinus betulus Hornbeam	Car b	Aero Plant	Carpinus Car b 1	160	1545887	7
		1.0105					
407	Carpinus betulus Hornbeam	Car b 1	Aero Plant	Carpinus Car b 1	160	1545891	7
408	Carpinus betulus Hornbeam	Car b	Aero Plant	Carpinus Car b 1	160	1545893	7
		1.0108					
409	Carpinus betulus Hornbeam	Car b	Aero Plant	Carpinus Car b 1	161	1545895	7
		1.0301					
410	Carpinus betulus Hornbeam	Car b	Aero Plant	Carpinus Car b 1	161	1545897	7
		1.0302					
411	Carpinus betulus Hornbeam	Unassigned	Aero Plant	Carpinus Car b 1	40	239735	7
412	Carpinus betulus Hornbeam	Unassigned	Aero Plant	Carpinus Car b 1	160	167472845	10
413	Carpinus betulus Hornbeam	Unassigned	Aero Plant	Carpinus Car b 1	160	167472837	10
414	Carpinus betulus Hornbeam	Unassigned	Aero Plant	Carpinus Car b 1	160	167472843	10
415	Carpinus betulus Hornbeam	Unassigned	Aero Plant	Carpinus Car b 1	160	167472841	10
416	Carpinus betulus Hornbeam	Unassigned	Aero Plant	Carpinus Car b 1	160	167472839	10
417	Carpinus betulus Hornbeam	Unassigned	Aero Plant	Carpinus Car b 1	80	1008578	12
418	Carpinus betulus Hornbeam	Unassigned	Aero Plant	Carpinus Car b 1	80	1008579	12
419	Carpinus betulus Hornbeam	Unassigned	Aero Plant	Carpinus Car b 1	80	1008580	12

420	Castanea sativa	European chestnut	Cas s 1	Aero Plant	Castanea Cas s 1	160	16555781	7
421	Castanea sativa	European chestnut	Unassigned	Aero Plant	Castanea Cas s 1	159	212291466	10
422	Castanea sativa	European chestnut	Unassigned	Aero Plant	Castanea Cas s 1	159	212291464	10
423	Castanea sativa	European chestnut	Unassigned	Aero Plant	Castanea Cas s 1	159	212291468	10
424	Castanea sativa	European chestnut	Unassigned	Aero Plant	Castanea Cas s 5	298	307159110	12
425	Castanea sativa	European chestnut	Cas s 5	Food Plant	Castanea Cas s 5	316	1359600	7
426	Cavia porcellus	Domestic guinea pig	Cav p 1	Aero Animal	Cavia Cav p 1	15	32469617	7
427	Cavia porcellus	Domestic guinea pig	Unassigned	Aero Animal	Cavia Cav p 2	170	325910590	12
428	Cavia porcellus	Domestic guinea pig	Unassigned	Aero Animal	Cavia Cav p 3 lipocalin	170	325910592	12
429	Chamaecyparis obtusa	Japanese cypress	Unassigned	Aero Plant	Chamaecyparis Cha o 1	375	9087163	9
430	Chamaecyparis obtusa	Japanese cypress	Unassigned	Aero Plant	Chamaecyparis Cha o 2	514	47606004	7
431	Chamaecyparis obtusa	Japanese cypress	Unassigned	Aero Plant	Chamaecyparis Cha o 2	419	114841683	8
432	Charybdis feriatius	Crab	Cha f 1	Food Animal	Charybdis Cha f 1	264	14285800	9
433	Chenopodium album	Pigweed	Unassigned	Aero Plant	Chenopodium Che a 1	168	47605504	9
434	Chenopodium album	Pigweed	Che a 2	Aero Plant	Chenopodium Che a 2	131	29465666	7
435	Chenopodium album	Pigweed	Unassigned	Aero Plant	Chenopodium Che a 2	133	238886048	11
436	Chenopodium album	Pigweed	Che a 3	Aero Plant	Chenopodium Che a 3	86	29465668	7
437	Chionoecetes opilio	Snow Crab	Unassigned	Food Animal	Chionoecetes tropomyosin	284	308191588	12
438	Chironomus kiiensis	Midge	Unassigned	Aero Insect	Chironomus Chi k 10	285	42559556	9
439	Chironomus thummi thummi	Midge	Chi t 1.01	Aero Insect	Chironomus Chi t 1	151	121219	7
440	Chironomus thummi thummi	Midge	Chi t 1.02	Aero Insect	Chironomus Chi t 1	151	121227	7
441	Chironomus thummi thummi	Midge	Chi t 2	Aero Insect	Chironomus Chi t 2	158	2506460	7
442	Chironomus thummi thummi	Midge	Chi t 3	Aero Insect	Chironomus Chi t 3	160	1707908	7

443	Chironomus thummi thummi	Midge	Chi t 4	Aero Insect	Chironomus Chi t 4	151	121256	17
444	Chironomus thummi thummi	Midge	Chi t 5	Aero Insect	Chironomus Chi t 5	162	2506461	17
445	Chironomus thummi thummi	Midge	Chi t 7	Aero Insect	Chironomus Chi t 7	161	56405052	17
446	Chironomus thummi thummi	Midge	Chi t 7	Aero Insect	Chironomus Chi t 7	161	121244	17
447	Chironomus thummi thummi	Midge	Chi t 7	Aero Insect	Chironomus Chi t 7	161	56405054	17
448	Chironomus thummi thummi	Midge	Chi t 7	Aero Insect	Chironomus Chi t 7	161	121248	17
449	Chironomus thummi thummi	Midge	Chi t 7	Aero Insect	Chironomus Chi t 7	162	121249	17
450	Chironomus thummi thummi	Midge	Chi t 8	Aero Insect	Chironomus Chi t 8	151	121237	17
451	Chironomus thummi thummi	Midge	Chi t 9	Aero Insect	Chironomus Chi t 9	151	121259	17
452	Citrus sinensis	Navel orange	Unassigned	Food Plant	Citrus Cit s 1	25	52782810	17
453	Citrus sinensis	Navel orange	Unassigned	Food Plant	Citrus Cit s 2	131	261260074	11
454	Citrus limon	Lemon	Unassigned	Food Plant	Citrus LTP Cit s 3	20	52783176	17
455	Citrus sinensis	Navel orange	Unassigned	Food Plant	Citrus LTP Cit s 3	20	52783177	17
456	Citrus sinensis	Navel orange	Cit s 3	Food Plant	Citrus LTP Cit s 3	91	50199132	17
457	Davidiella tassiana	Fungus	Unassigned	Aero Fungi	Cladosporium / Davidiella Cla h 10	496	108935817	18
458	Davidiella tassiana	Fungus	Cla h 5	Aero Fungi	Cladosporium / Davidiella Cla h 5	111	1173074	17
459	Davidiella tassiana	Fungus	Cla h 5	Aero Fungi	Cladosporium / Davidiella Cla h 5	111	21542440	17
460	Davidiella tassiana	Fungus	Cla h 6	Aero Fungi	Cladosporium / Davidiella Cla h 6	440	467660	17
461	Davidiella tassiana	Fungus	Cla h 6	Aero Fungi	Cladosporium / Davidiella Cla h 6	440	6015094	17
462	Davidiella tassiana	Fungus	Cla h 7	Aero Fungi	Cladosporium / Davidiella Cla h 7	204	1168970	17
463	Davidiella tassiana	Fungus	Unassigned	Aero Fungi	Cladosporium / Davidiella Cla h 8	267	85701146	17
464	Davidiella tassiana	Fungus	Unassigned	Aero Fungi	Cladosporium / Davidiella Cla h 9 vacuolar serine	518	60116876	10
465	Davidiella tassiana	Fungus	Unassigned	Aero Fungi	Cladosporium / Davidiella Heat shock 70 kDa protei	643	729764	17
466	Davidiella tassiana	Fungus	Unassigned	Aero Fungi	Cladosporium / Davidiella Hydrophobin	105	22796153	17

467	Davidiella tassiana	Fungus	Unassigned	Aero Fungi	Cladosporium / Davidiella Putative nuclear transpo	125	21748151	7
468	Cladosporium cladosporioides		Unassigned	Aero Fungi	Cladosporium Cla c 9 Davidiella	388	148361511	11
469	Cochliobolus lunatus		Unassigned	Aero Fungi	Cochliobolus (Curvularia) Cur l 3 * ver 10	108	20137645	8
470	Cochliobolus lunatus		Cur l 2.01	Aero Fungi	Cochliobolus (Curvularia) enolase Cur l 2.01	440	14585753	8
471	Coprinus comatus	Shaggy mane	Cop c 1	Food Fungi	Coprinus Cop c 1	81	4538529	7
472	Corylus avellana	European hazelnut	Cor a 1.0103	Aero Plant	Corylus Cor a 1	160	22684	7
473	Corylus avellana	European hazelnut	Cor a 1.0104	Aero Plant	Corylus Cor a 1	160	22686	7
474	Corylus avellana	European hazelnut	Cor a 1.0102	Aero Plant	Corylus Cor a 1	160	22690	7
475	Corylus avellana	European hazelnut	Cor a 1.0201	Aero Plant	Corylus Cor a 1	160	1321731	7
476	Corylus avellana	European hazelnut	Cor a 1.0301	Aero Plant	Corylus Cor a 1	160	1321733	7
477	Corylus avellana	European hazelnut	Cor a I	Aero Plant	Corylus Cor a 1	160	584968	7
478	Corylus avellana	European hazelnut	Cor a 1.0401	Food Plant	Corylus Cor a 1	161	5726304	7
479	Corylus avellana	European hazelnut	Cor a 1.0402	Food Plant	Corylus Cor a 1	161	11762102	7
480	Corylus avellana	European hazelnut	Cor a 1.0403	Food Plant	Corylus Cor a 1	161	11762104	7
481	Corylus avellana	European hazelnut	Cor a 1.0404	Food Plant	Corylus Cor a 1	161	11762106	7
482	Corylus avellana	European hazelnut	Cor a 10	Aero Plant	Corylus Cor a 10	668	10944737	7
483	Corylus avellana	European hazelnut	Cor a 11	Food Plant	Corylus Cor a 11	448	19338630	7
484	Corylus avellana	European hazelnut	Unassigned	Food Plant	Corylus Cor a 14 2S albumin	147	226437844	11
485	Corylus avellana	European hazelnut	Cor a 2	Aero Plant	Corylus Cor a 2	131	12659206	7
486	Corylus avellana	European hazelnut	Cor a 2	Aero Plant	Corylus Cor a 2	131	12659208	7
487	Corylus avellana	European hazelnut	Cor a 8	Food Plant	Corylus Cor a 8	115	13507262	7
488	Corylus avellana	European hazelnut	Cor a 9	Food Plant	Corylus Cor a 9	515	18479082	7

489	Corylus avellana	European hazelnut	Unassigned	Food Plant	Corylus Oleosin	140	29170509	7
490	Crangon crangon		Unassigned	Food Animal	Crangon Cra c 1 tropomyosin	284	238477263	12
491	Crangon crangon		Unassigned	Food Animal	Crangon Cra c 2 arginine kinase	356	238477265	12
492	Crangon crangon		Unassigned	Food Animal	Crangon Cra c 4 sarcoplasmic calcium-binding prote	193	238477327	12
493	Crangon crangon		Unassigned	Food Animal	Crangon Cra c 5 myosin light chain	153	238477331	12
494	Crangon crangon		Unassigned	Food Animal	Crangon Cra c 6 troponin C	150	238477333	12
495	Crangon crangon		Unassigned	Food Animal	Crangon Cra c 8 triosephosphate isomerase	249	238477329	12
496	Crassostrea gigas	American oyster	Unassigned	Food Animal	Crassostrea Tropomyosin	233	15419048	7
497	Crassostrea gigas	American oyster	Unassigned	Food Animal	Crassostrea Tropomyosin	284	219806594	10
498	Crassostrea virginica	Eastern oyster	Unassigned	Food Animal	Crassostrea Tropomyosin	160	3668408	7
499	Crocus sativus	Saffron crocus	Unassigned	Aero Plant	Crocus profilin Cro s 2	131	58700651	7
500	Cryptomeria japonica	Japanese cedar	Unassigned	Aero Plant	Cryptomeria class IV chitinase	281	56550550	7
501	Cryptomeria japonica	Japanese cedar	Cry j 1	Aero Plant	Cryptomeria Cry j 1	374	1173367	7
502	Cryptomeria japonica	Japanese cedar	Cry j 1	Aero Plant	Cryptomeria Cry j 1	374	19570315	7
503	Cryptomeria japonica	Japanese cedar	Unassigned	Aero Plant	Cryptomeria Cry j 1	374	493634	8
504	Cryptomeria japonica	Japanese cedar	Cry j 2	Aero Plant	Cryptomeria Cry j 2	514	1171004	7
505	Cryptomeria japonica	Japanese cedar	Cry j 2	Aero Plant	Cryptomeria Cry j 2	514	24898904	7
506	Cryptomeria japonica	Japanese cedar	Cry j 2	Aero Plant	Cryptomeria Cry j 2	514	24898906	7
507	Cryptomeria japonica	Japanese cedar	Cry j 2	Aero Plant	Cryptomeria Cry j 2	514	24898908	7
508	Cryptomeria japonica	Japanese cedar	Unassigned	Aero Plant	Cryptomeria Cry j 2	514	114841607	8
509	Cryptomeria japonica	Japanese cedar	Unassigned	Aero Plant	Cryptomeria Cry j 2	514	114841617	8
510	Cryptomeria japonica	Japanese cedar	Unassigned	Aero Plant	Cryptomeria Cry j 2	514	114841629	8

511	Cryptomeria japonica	Japanese cedar	Unassigned	Aero Plant	Cryptomeria Cry j 2	514	114841635	8
512	Cryptomeria japonica	Japanese cedar	Unassigned	Aero Plant	Cryptomeria Cry j 2	514	114841641	8
513	Cryptomeria japonica	Japanese cedar	Unassigned	Aero Plant	Cryptomeria Cry j 2	514	114841653	8
514	Cryptomeria japonica	Japanese cedar	Unassigned	Aero Plant	Cryptomeria Cry j 2	514	114841657	8
515	Cryptomeria japonica	Japanese cedar	Unassigned	Aero Plant	Cryptomeria Cry j 2	514	114841663	8
516	Cryptomeria japonica	Japanese cedar	Unassigned	Aero Plant	Cryptomeria Cry j 2	514	114841665	8
517	Cryptomeria japonica	Japanese cedar	Unassigned	Aero Plant	Cryptomeria Cry j 2	514	114841671	8
518	Cryptomeria japonica	Japanese cedar	Unassigned	Aero Plant	Cryptomeria Cry j 2	65	123299282	19
519	Cryptomeria japonica	Japanese cedar	Unassigned	Aero Plant	Cryptomeria Isoflavone reductase-like protein	306	19847822	17
520	Cryptomeria japonica	Japanese cedar	Unassigned	Aero Plant	Cryptomeria pollen allergen CJP-8	165	291621332	12
521	Cryptomeria japonica	Japanese cedar	Unassigned	Aero Plant	Cryptomeria pollen allergen CPA63	472	293329689	12
522	Cryptomeria japonica	Japanese cedar	Unassigned	Aero Plant	Cryptomeria thaumatin like Cry j 3.8	225	139002766	8
523	Cucumis melo	Muskmelon	Unassigned	Food Plant	Cucumis Cuc m 1	731	71153243	19
524	Cucumis melo	Muskmelon	Cuc m 2	Food Plant	Cucumis Cuc m 2	131	31559374	17
525	Cucumis melo	Muskmelon	Cuc m 2	Food Plant	Cucumis Cuc m 2	131	58263793	17
526	Cucumis melo var. reticulatus	Netted muskmelon	Cuc m 2	Food Plant	Cucumis Cuc m 2	131	57021110	17
527	Cucumis melo	Muskmelon	Cuc m 3	Food Plant	Cucumis Cuc m 3	21	46396596	17
528	Cucumis melo	Muskmelon	Cuc m 3	Food Plant	Cucumis Cuc m 3	10	46396597	17
529	Cucumis melo	Muskmelon	Cuc m 3	Food Plant	Cucumis Cuc m 3	10	46396598	17
530	Cucumis melo var. inodorus	Muskmelon	Unassigned	Food Plant	Cucumis Cuc m 3	151	171464770	19
531	Cupressus arizonica	Arizona Cypress	Cup a 1	Aero Plant	Cupressus Cup a 1	367	19069497	17
532	Cupressus arizonica	Arizona Cypress	Unassigned	Aero Plant	Cupressus Cup a 1	347	118197955	8
533	Cupressus arizonica	Arizona Cypress	Unassigned	Aero Plant	Cupressus Cup a 1	346	9087167	19
534	Cupressus sempervirens	Mediterranean Cypress	Cup s 1.0101	Aero Plant	Cupressus Cup a 1	367	8101711	17

535	Cupressus sempervirens	Mediterranean Cypress	Cup s 1.0102	Aero Plant	Cupressus Cup a 1	367	8101713	17
536	Cupressus sempervirens	Mediterranean Cypress	Cup s 1.0103	Aero Plant	Cupressus Cup a 1	367	8101715	17
537	Cupressus sempervirens	Mediterranean Cypress	Cup s 1.0104	Aero Plant	Cupressus Cup a 1	367	8101717	17
538	Cupressus sempervirens	Mediterranean Cypress	Cup s 1.0105	Aero Plant	Cupressus Cup a 1	367	8101719	17
539	Cupressus arizonica	Arizona Cypress	Unassigned	Aero Plant	Cupressus Cup s 3	199	9929163	17
540	Cupressus sempervirens	Mediterranean Cypress	Unassigned	Aero Plant	Cupressus Cup s 3	225	38456230	17
541	Cupressus sempervirens	Mediterranean Cypress	Unassigned	Aero Plant	Cupressus Cup s 3	225	38456228	17
542	Cupressus arizonica	Arizona Cypress	Unassigned	Aero Plant	Cupressus putative allergen Cup a 4	165	261865475	11
543	Cynodon dactylon	Bermuda grass	Cyn d 1	Aero Plant	Cynodon Cyn d 1	25	451274	17
544	Cynodon dactylon	Bermuda grass	Cyn d 1	Aero Plant	Cynodon Cyn d 1	38	451275	17
545	Cynodon dactylon	Bermuda grass	Cyn d 1	Aero Plant	Cynodon Cyn d 1	34	691726	17
546	Cynodon dactylon	Bermuda grass	Cyn d 1.0204	Aero Plant	Cynodon Cyn d 1	244	10314021	17
547	Cynodon dactylon	Bermuda grass	Cyn d 1	Aero Plant	Cynodon Cyn d 1	246	14423757	17
548	Cynodon dactylon	Bermuda grass	Cyn d 1.0201	Aero Plant	Cynodon Cyn d 1	244	15384338	17
549	Cynodon dactylon	Bermuda grass	Cyn d 1.0202	Aero Plant	Cynodon Cyn d 1	262	16076693	17
550	Cynodon dactylon	Bermuda grass	Cyn d 1	Aero Plant	Cynodon Cyn d 1	262	16076695	17
551	Cynodon dactylon	Bermuda grass	Cyn d 1.0203	Aero Plant	Cynodon Cyn d 1	262	16076697	17
552	Cynodon dactylon	Bermuda grass	Cyn d 12	Aero Plant	Cynodon Cyn d 12	131	2154730	17
553	Cynodon dactylon	Bermuda grass	Unassigned	Aero Plant	Cynodon Cyn d 7	71	1247373	17
554	Cynodon dactylon	Bermuda grass	Unassigned	Aero Plant	Cynodon Cyn d 7	73	1247375	17
555	Cynodon dactylon	Bermuda grass	Cyn d 7	Aero Plant	Cynodon Cyn d 7	82	1871507	17
556	Cyprinus carpio	Carp	Unassigned	Food Animal	Cyprinus Parvalbumin	109	17977825	17
557	Cyprinus carpio	Carp	Unassigned	Food Animal	Cyprinus Parvalbumin	109	17977827	17
558	Dactylis glomerata	Orchard grass	Dac g 1	Aero Plant	Dactylis Dac g 1	264	18093991	17
559	Dactylis glomerata	Orchard grass	Unassigned	Aero Plant	Dactylis Dac g 1	240	33149333	17
560	Dactylis glomerata	Orchard grass	Dac g 2	Aero Plant	Dactylis Dac g 2	196	1093120	17

561	Dactylis glomerata	Orchard grass	Dac g 2	Aero Plant	Dactylis Dac g 2	122	4007040	7
562	Dactylis glomerata	Orchard grass	Unassigned	Aero Plant	Dactylis Dac g 3	96	14423759	8
563	Dactylis glomerata	Orchard grass	Unassigned	Aero Plant	Dactylis Dac g 4	12	32363464	7
564	Dactylis glomerata	Orchard grass	Unassigned	Aero Plant	Dactylis Dac g 4	11	32363465	7
565	Dactylis glomerata	Orchard grass	Unassigned	Aero Plant	Dactylis Dac g 4	17	32363466	7
566	Dactylis glomerata	Orchard grass	Unassigned	Aero Plant	Dactylis Dac g 4	15	32363467	7
567	Dactylis glomerata	Orchard grass	Dac g 5	Aero Plant	Dactylis Dac g 5	290	14423124	7
568	Dactylis glomerata	Orchard grass	Dac g 5	Aero Plant	Dactylis Dac g 5	265	18093971	7
569	Daucus carota	Carrot	Dau c 1.0101	Food Plant	Daucus Dau c 1	168	1335877	7
570	Daucus carota	Carrot	Dau c 1.0102	Food Plant	Daucus Dau c 1	154	1663522	7
571	Daucus carota	Carrot	Dau c 1.0103	Food Plant	Daucus Dau c 1	154	2154732	7
572	Daucus carota	Carrot	Dau c 1.0104	Food Plant	Daucus Dau c 1	154	2154734	7
573	Daucus carota	Carrot	Dau c 1.0201	Food Plant	Daucus Dau c 1	154	18652047	7
574	Daucus carota	Carrot	Unassigned	Food Plant	Daucus Dau c 1	154	19912791	7
575	Daucus carota	Carrot	Dau c 1.0105	Food Plant	Daucus Dau c 1	154	8928058	9
576	Daucus carota	Carrot	Dau c 1.0301	Food Plant	Daucus Dau c 1	154	302379147	12
577	Daucus carota	Carrot	Unassigned	Food Plant	Daucus Dau c 1	154	302379149	12
578	Daucus carota	Carrot	Unassigned	Food Plant	Daucus Dau c 1	154	302379151	12
579	Daucus carota	Carrot	Unassigned	Food Plant	Daucus Dau c 1	154	302379153	12
580	Daucus carota	Carrot	Unassigned	Food Plant	Daucus Dau c 1	154	302379155	12
581	Daucus carota	Carrot	Unassigned	Food Plant	Daucus Dau c 1	154	302379157	12
582	Daucus carota	Carrot	Unassigned	Food Plant	Daucus Dau c 1	154	302379159	12
583	Daucus carota	Carrot	Unassigned	Food Plant	Daucus Dau c 4	134	47606043	10
584	Dermatophagoides farinae	House dust mite	Unassigned	Aero Mite	Dermatophagoides Der f 13	131	99031759	7
585	Dermatophagoides farinae	House dust mite	Der f 16	Aero Mite	Dermatophagoides Der f 16	480	21591547	7
586	Dermatophagoides farinae	House dust mite	Der f 1	Aero Mite	Dermatophagoides Der p 1 Der f 1	321	730035	7

587	Dermatophagoides  farinae	House dust  mite	Der f 1	Aero Mite	Dermatophagoides  Der p 1 Der f 1	321	27530349	7
588	Dermatophagoides  farinae	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 1 Der f 1	276	76097507	7
589	Dermatophagoides  farinae	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 1 Der f 1	321	156106765	9
590	Dermatophagoides  farinae	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 1 Der f 1	263	37958161	12
591	Dermatophagoides  microceras	House dust  mite	Der m 1	Aero Mite	Dermatophagoides  Der p 1 Der f 1	30	127205	7
592	Dermatophagoides  pteronysinus	House dust  mite	Der p 1	Aero Mite	Dermatophagoides  Der p 1 Der f 1	320	730036	7
593	Dermatophagoides  pteronysinus	House dust  mite	Der p 1	Aero Mite	Dermatophagoides  Der p 1 Der f 1	222	21725560	7
594	Dermatophagoides  pteronysinus	House dust  mite	Der p 1	Aero Mite	Dermatophagoides  Der p 1 Der f 1	222	21725562	7
595	Dermatophagoides  pteronysinus	House dust  mite	Der p 1	Aero Mite	Dermatophagoides  Der p 1 Der f 1	222	21725564	7
596	Dermatophagoides  pteronysinus	House dust  mite	Der p 1	Aero Mite	Dermatophagoides  Der p 1 Der f 1	222	21725566	7
597	Dermatophagoides  pteronysinus	House dust  mite	Der p 1	Aero Mite	Dermatophagoides  Der p 1 Der f 1	222	21725568	7
598	Dermatophagoides  pteronysinus	House dust  mite	Der p 1	Aero Mite	Dermatophagoides  Der p 1 Der f 1	222	21725570	7
599	Dermatophagoides  pteronysinus	House dust  mite	Der p 1	Aero Mite	Dermatophagoides  Der p 1 Der f 1	222	21725572	7
600	Dermatophagoides  pteronysinus	House dust  mite	Der p 1	Aero Mite	Dermatophagoides  Der p 1 Der f 1	222	21725574	7
601	Dermatophagoides  pteronysinus	House dust  mite	Der p 1	Aero Mite	Dermatophagoides  Der p 1 Der f 1	222	21725576	7
602	Dermatophagoides  pteronysinus	House dust  mite	Der p 1	Aero Mite	Dermatophagoides  Der p 1 Der f 1	222	21725578	7
603	Dermatophagoides  pteronysinus	House dust  mite	Der p 1	Aero Mite	Dermatophagoides  Der p 1 Der f 1	222	21725580	7
604	Dermatophagoides  pteronysinus	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 1 Der f 1	216	61608445	7
605	Dermatophagoides  pteronysinus	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 1 Der f 1	222	83754033	7
606	Dermatophagoides  pteronysinus	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 1 Der f 1	211	1460058	8
607	Dermatophagoides  pteronysinus	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 1 Der f 1	223	157696052	9
608	Dermatophagoides  pteronysinus	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 1 Der f 1	222	223365887	10
609	Dermatophagoides  pteronysinus	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 1 Der f 1	320	195933901	10



633	Dermatophagoides  farinae	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 2 / Der f 2	140	37958157	12
634	Dermatophagoides  pteronyssinus	House dust  mite	Der p 2	Aero Mite	Dermatophagoides  Der p 2 / Der f 2	146	1352237	17
635	Dermatophagoides  pteronyssinus	House dust  mite	Der p 2	Aero Mite	Dermatophagoides  Der p 2 / Der f 2	129	21465915	17
636	Dermatophagoides  pteronyssinus	House dust  mite	Der p 2	Aero Mite	Dermatophagoides  Der p 2 / Der f 2	129	21725582	17
637	Dermatophagoides  pteronyssinus	House dust  mite	Der p 2	Aero Mite	Dermatophagoides  Der p 2 / Der f 2	129	21725584	17
638	Dermatophagoides  pteronyssinus	House dust  mite	Der p 2	Aero Mite	Dermatophagoides  Der p 2 / Der f 2	129	21725586	17
639	Dermatophagoides  pteronyssinus	House dust  mite	Der p 2	Aero Mite	Dermatophagoides  Der p 2 / Der f 2	129	21725588	17
640	Dermatophagoides  pteronyssinus	House dust  mite	Der p 2	Aero Mite	Dermatophagoides  Der p 2 / Der f 2	129	21725590	17
641	Dermatophagoides  pteronyssinus	House dust  mite	Der p 2	Aero Mite	Dermatophagoides  Der p 2 / Der f 2	129	21725592	17
642	Dermatophagoides  pteronyssinus	House dust  mite	Der p 2	Aero Mite	Dermatophagoides  Der p 2 / Der f 2	129	21725594	17
643	Dermatophagoides  pteronyssinus	House dust  mite	Der p 2	Aero Mite	Dermatophagoides  Der p 2 / Der f 2	129	21725596	17
644	Dermatophagoides  pteronyssinus	House dust  mite	Der p 2	Aero Mite	Dermatophagoides  Der p 2 / Der f 2	129	21725600	17
645	Dermatophagoides  pteronyssinus	House dust  mite	Der p 2	Aero Mite	Dermatophagoides  Der p 2 / Der f 2	129	21725602	17
646	Dermatophagoides  pteronyssinus	House dust  mite	Der p 2	Aero Mite	Dermatophagoides  Der p 2 / Der f 2	129	21725604	17
647	Dermatophagoides  pteronyssinus	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 2 / Der f 2	129	76097509	17
648	Dermatophagoides  pteronyssinus	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 2 / Der f 2	146	99644635	17
649	Dermatophagoides  pteronyssinus	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 2 / Der f 2	130	110560872	19
650	Dermatophagoides  pteronyssinus	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 2 / Der f 2	129	157829757	19
651	Dermatophagoides  pteronyssinus	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 2 / Der f 2	145	164415595	19
652	Dermatophagoides  pteronyssinus	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 2 / Der f 2	129	256095984	11
653	Dermatophagoides  siboney	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 2 / Der f 2	146	86450747	17
654	Dermatophagoides  pteronyssinus	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 21	140	85687540	17
655	Dermatophagoides  farinae	House dust  mite	Der f 3	Aero Mite	Dermatophagoides  Der p 3 / Der f 3	232	1314736	17

656	Dermatophagoides  farinae	House dust  mite	Der f 3	Aero Mite	Dermatophagoides  Der p 3 / Der f 3	259	2507248	17
657	Dermatophagoides  farinae	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 3 / Der f 3	259	163638970	19
658	Dermatophagoides  farinae	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 3 / Der f 3	259	218203816	10
659	Dermatophagoides  farinae	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 3 / Der f 3	259	218203818	10
660	Dermatophagoides  pteronysinus	House dust  mite	Der p 3	Aero Mite	Dermatophagoides  Der p 3 / Der f 3	261	511476	17
661	Dermatophagoides  pteronysinus	House dust  mite	Der p 4	Aero Mite	Dermatophagoides  Der p 4	496	5059162	17
662	Dermatophagoides  pteronysinus	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 4	19	1351935	17
663	Dermatophagoides  pteronysinus	House dust  mite	Der p 5	Aero Mite	Dermatophagoides  Der p 5	132	1352238	17
664	Dermatophagoides  pteronysinus	House dust  mite	Der p 5	Aero Mite	Dermatophagoides  Der p 5	132	913285	17
665	Dermatophagoides  pteronysinus	House dust  mite	Der p 5	Aero Mite	Dermatophagoides  Der p 5	132	28798085	17
666	Dermatophagoides  farinae	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 6 / Der f 6	279	14424450	17
667	Dermatophagoides  farinae	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 6 / Der f 6	20	404371	17
668	Dermatophagoides  farinae	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 6 / Der f 6	279	218203826	10
669	Dermatophagoides  farinae	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 6 / Der f 6	279	218203828	10
670	Dermatophagoides  pteronysinus	House dust  mite	Der p 6	Aero Mite	Dermatophagoides  Der p 6 / Der f 6	20	1352239	17
671	Dermatophagoides  farinae	House dust  mite	Der f 7	Aero Mite	Dermatophagoides  Der p 7 / Der f 7	213	2498299	17
672	Dermatophagoides  farinae	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 7 / Der f 7	213	37958165	18
673	Dermatophagoides  farinae	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 7 / Der f 7	213	218203832	10
674	Dermatophagoides  pteronysinus	House dust  mite	Der p 7	Aero Mite	Dermatophagoides  Der p 7 / Der f 7	215	10189811	17
675	Dermatophagoides  pteronysinus	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 7 / Der f 7	215	1352240	19
676	Dermatophagoides  pteronysinus	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 8	219	60920878	17
677	Dermatophagoides  pteronysinus	House dust  mite	Unassigned	Aero Mite	Dermatophagoides  Der p 8	219	1170095	19
678	Dermatophagoides  farinae	House dust  mite	Der f 18	Aero Mite	Dermatophagoides  farinae Der f 18	462	27550039	17

					Der p chitinase			
679	Dermatophagoides pteronyssinus	House dust mite	Unassigned	Aero Mite	Dermatophagoides farinae Der f 18 Der p chitinase	462	67975085	7
680	Dermatophagoides farinae	House dust mite	Unassigned	Aero Mite	Dermatophagoides farinae Der f 21 Chew	136	60679572	9
681	Dermatophagoides farinae	House dust mite	Unassigned	Aero Mite	Dermatophagoides farinae Der f 21 Chew	136	140089314	9
682	Dermatophagoides farinae	House dust mite	Unassigned	Aero Mite	Dermatophagoides farinae Der f 21 Chew	136	140089316	9
683	Dermatophagoides farinae	House dust mite	Unassigned	Aero Mite	Dermatophagoides farinae Der f 21 Chew	136	140089320	9
684	Dermatophagoides farinae	House dust mite	Unassigned	Aero Mite	Dermatophagoides farinae Der f 21 Chew	136	140089322	9
685	Dermatophagoides farinae	House dust mite	Unassigned	Aero Mite	Dermatophagoides farinae Der f 21 Chew	136	140089324	9
686	Dermatophagoides farinae	House dust mite	Unassigned	Aero Mite	Dermatophagoides farinae Der f 21 Chew	136	140089326	9
687	Dolichovespula maculata	Whiteface hornet	Dol m 1	Venom or Salivary	Dolichovespula Dol m 1	317	548449	7
688	Dolichovespula maculata	Whiteface hornet	Dol m 1	Venom or Salivary	Dolichovespula Dol m 1	303	1709542	7
689	Dolichovespula maculata	Whiteface hornet	Dol m 2	Venom or Salivary	Dolichovespula Dol m 2	331	1346322	7
690	Dolichovespula arenaria	Yellow jacket	Dol a 5	Venom or Salivary	Dolichovespula Venom allergen 5	203	465052	7
691	Dolichovespula maculata	Whiteface hornet	Dol m 5	Venom or Salivary	Dolichovespula Venom allergen 5	227	137395	7
692	Dolichovespula maculata	Whiteface hornet	Dol m 5	Venom or Salivary	Dolichovespula Venom allergen 5	215	549186	7
693	Epicoccum nigrum	Fungus	Unassigned	Aero Fungi	Epicoccum Epi p 1	18	24636820	9
694	Equus caballus	Horse	Equ c 1	Aero Animal	Equus Equ c 1	187	3121758	7
695	Equus caballus	Horse	Equ c 2.0101	Aero Animal	Equus Equ c 2	29	3121755	7
696	Equus caballus	Horse	Equ c 2.0102	Aero Animal	Equus Equ c 2	19	3121756	7
697	Equus caballus	Horse	Unassigned	Aero Animal	Equus Equ c 3	607	543794	9
698	Equus caballus	Horse	Unassigned	Aero Animal	Equus Equ c 4	228	38258932	8

699	Equus caballus	Horse	Unassigned	Aero	Equus Equ c 4	228	152031631	9
				Animal				
700	Erimacrus		Unassigned	Food	Erimacrus	284	125995169	8
	lisenbeckii			Animal	tropomyosin			
701	Erimacrus		Unassigned	Food	Erimacrus	284	125995171	8
	lisenbeckii			Animal	tropomyosin			
702	Euphausia		Unassigned	Food	Euphausia	284	156712754	9
	pacificica			Animal				
703	Euphausia		Unassigned	Food	Euphausia	284	156712752	9
	superba			Animal				
704	Euroglyphus	House dust	Eur m	Aero Mite	Euroglyphus Eur m 2	135	3941386	7
	lmaynei	mite	2.0102					
705	Euroglyphus	House dust	Eur m 2	Aero Mite	Euroglyphus Eur m 2	145	14423649	7
	lmaynei	mite						
706	Fagopyrum	Buckwheat	Unassigned	Food Plant	Fagopyrum BW 16kDa	127	61970231	7
	lesculentum				allergen			
707	Fagopyrum	Buckwheat	Unassigned	Food Plant	Fagopyrum BW 16kDa	149	83416591	7
	lesculentum				allergen			
708	Fagopyrum	Buckwheat	Unassigned	Food Plant	Fagopyrum BW 16kDa	149	320445237	12
	tataricum				allergen			
709	Fagopyrum	Buckwheat	Unassigned	Food Plant	Fagopyrum BW 8 kDa	133	17907758	7
	lesculentum				protein			
710	Fagopyrum	Buckwheat	Unassigned	Food Plant	Fagopyrum BW 8 kDa	133	144228127	8
	tataricum				protein			
711	Fagopyrum	Buckwheat	Unassigned	Food Plant	Fagopyrum	565	29839254	9
	lesculentum				Legumin-like			
					protein			
712	Fagopyrum	Buckwheat	Unassigned	Food Plant	Fagopyrum	504	29839255	9
	lesculentum				Legumin-like			
					protein			
713	Fagopyrum	Buckwheat	Unassigned	Food Plant	Fagopyrum	538	29839419	9
	lesculentum				Legumin-like			
					protein			
714	Fagopyrum	Buckwheat	Unassigned	Food Plant	Fagopyrum	191	6979766	7
	gracilipes				Legumin-like			
					protein			
715	Fagopyrum	Buckwheat	Unassigned	Food Plant	Fagopyrum	515	113200131	9
	tataricum				Legumin-like			
					protein			
716	Fagopyrum	Buckwheat	Unassigned	Food Plant	Fagopyrum	136	146217148	9
	lesculentum				vicilin-like			
					protein			
717	Fagus sylvatica	European	Unassigned	Aero Plant	Fagus Fag s 1	160	212291472	10
	Beech							
718	Fagus sylvatica	European	Fag s 1	Aero Plant	Fagus Fag s 1	160	212291470	10
	Beech							
719	Fagus sylvatica	European	Unassigned	Aero Plant	Fagus Fag s 1	160	212291474	10
	Beech							

720	Farfantepenaeus  aztecus	Brown shrimp	Pen a 1	Food  Animal	Farfantepenaeus Pen  a 1	284	73532979	7
721	Felis catus	Cat	Fel d 1	Aero  Animal	Felis Fel d 1 Chain  1	88	1364212	7
722	Felis catus	Cat	Fel d 1	Aero  Animal	Felis Fel d 1 Chain  1	92	1364213	7
723	Felis catus	Cat	Fel d 1	Aero  Animal	Felis Fel d 1 Chain  1	92	1169665	7
724	Felis catus	Cat	Fel d 1	Aero  Animal	Felis Fel d 1 Chain  1	92	163825	7
725	Felis catus	Cat	Unassigned	Aero  Animal	Felis Fel d 1 Chain  1	88	114326420	18
726	Felis catus	Cat	Fel d 1	Aero  Animal	Felis Fel d 1 chain  2	109	232086	7
727	Felis catus	Cat	Unassigned	Aero  Animal	Felis Fel d 1 chain  2	107	395407	8
728	Felis catus	Cat	Unassigned	Aero  Animal	Felis Fel d 2	608	1351908	9
729	Felis catus	Cat	Unassigned	Aero  Animal	Felis Fel d 3	98	47605720	9
730	Felis catus	Cat	Unassigned	Aero  Animal	Felis Fel d 4	186	75062228	8
731	Felis catus	Cat	Unassigned	Aero  Animal	Felis Fel d 7 von  Ebner gland protein	180	301072397	12
732	Felis catus	Cat	Unassigned	Aero  Animal	Felis Fel d 8  latherin-like	228	303387468	12
733	Schedonorus  arundinaceus	Tall fescue	Unassigned	Aero Plant	Festuca group 1  allergen	35	75139991	7
734	Schedonorus  arundinaceus	Tall fescue	Unassigned	Aero Plant	Festuca group 1  allergen	17	320610	7
735	Schedonorus  arundinaceus	Tall fescue	Unassigned	Aero Plant	Festuca group 1  allergen	20	320611	7
736	Forcipomyia  taiwana	biting midges	Unassigned	Venom or  Salivary	Forcipomyia For t 1	118	188572341	10
737	Forcipomyia  taiwana	biting midges	Unassigned	Venom or  Salivary	Forcipomyia For t 2	325	188572343	10
738	Fragaria x  ananassa	Strawberry	Fra a 1	Food Plant	Fragaria Fra a 1	14	60389904	7
739	Fragaria x  ananassa	Strawberry	Fra a 1	Food Plant	Fragaria Fra a 1	74	60389905	7
740	Fragaria x  ananassa	Strawberry	Fra a 1	Food Plant	Fragaria Fra a 1	160	90185692	7
741	Fragaria x  ananassa	Strawberry	Fra a 1	Food Plant	Fragaria Fra a 1	159	90185688	7
742	Fragaria x  ananassa	Strawberry	Fra a 1	Food Plant	Fragaria Fra a 1	160	90185684	7

743	Fragaria x ananassa	Strawberry	Fra a 1	Food Plant	Fragaria Fra a 1	160	90185682	7
744	Fragaria x ananassa	Strawberry	Fra a 1	Food Plant	Fragaria Fra a 1	160	88082485	7
745	Fraxinus excelsior	European ash	Fra e 1	Aero Plant	Fraxinus Fra e 1	146	34978692	7
746	Fraxinus excelsior	European ash	Fra e 1	Aero Plant	Fraxinus Fra e 1	145	56122438	7
747	Fraxinus excelsior	European ash	Fra e 1	Aero Plant	Fraxinus Fra e 1	145	33327133	7
748	Fulvia mutica		Unassigned	Food Animal	Fulvia tropomyosin	284	219806596	10
749	Fusarium culmorum	Fungus	Unassigned	Aero Fungi	Fusarium claimed Fus c 3	450	25361513	7
750	Fusarium culmorum	Fungus	Unassigned	Aero Fungi	Fusarium Fus c 1	109	41688715	10
751	Fusarium culmorum	Fungus	Unassigned	Aero Fungi	Fusarium Fus c 2	121	52783462	9
752	Gadus callarias	Baltic cod	Gad c 1	Food Animal	Gadus Gad c 1	113	131112	7
753	Gadus morhua	Atlantic cod	Unassigned	Food Animal	Gadus Gad c 1	109	14531014	7
754	Gadus morhua	Atlantic cod	Unassigned	Food Animal	Gadus Gad c 1	109	14531016	7
755	Gadus morhua	Atlantic cod	Unassigned	Food Animal	Gadus Gad c 1	109	148356691	9
756	Gadus morhua	Atlantic cod	Unassigned	Food Animal	Gadus Gad c 1	109	148356693	9
757	Gallus gallus	Chicken	Gal d 1	Food Animal	Gallus Gal d 1	210	124757	7
758	Gallus gallus	Chicken	Unassigned	Food Animal	Gallus Gal d 1	208	162952006	9
759	Gallus gallus	Chicken	Unassigned	Food Animal	Gallus Gal d 1	210	209979542	10
760	Gallus gallus	Chicken	Gal d 2	Food Animal	Gallus Gal d 2	155	63052	7
761	Gallus gallus	Chicken	Gal d 2	Food Animal	Gallus Gal d 2	386	129293	7
762	Gallus gallus	Chicken	Gal d 2	Food Animal	Gallus Gal d 2	386	808969	7
763	Gallus gallus	Chicken	Gal d 2	Food Animal	Gallus Gal d 2	385	15826578	7
764	Gallus gallus	Chicken	Unassigned	Food Animal	Gallus Gal d 2	385	34811333	7
765	Gallus gallus	Chicken	Gal d 3	Food Animal	Gallus Gal d 3	705	757851	7

766	Gallus gallus	Chicken	Gal d 3	Food Animal	Gallus Gal d 3	705	1351295	7
767	Gallus gallus	Chicken	Gal d 4	Food Animal	Gallus Gal d 4	147	126608	7
768	Gallus gallus	Chicken	Gal d 4	Food Animal	Gallus Gal d 4	24	212279	7
769	Gallus gallus	Chicken	Unassigned	Food Animal	Gallus Gal d 5	615	113575	9
770	Gallus gallus	Chicken	Unassigned	Food Animal	Gallus parvalbumin	110	225877920	10
771	Gibberella zeae PH-1	Fungus	Unassigned	Aero Fungi	Gibberella 60S acidic ribosomal protein P2	109	46122455	7
772	Glossina morsitans morsitans	Tsetse fly	Unassigned	Venom or Salivary	Glossina Glo m 5	258	289740263	11
773	Glossina morsitans morsitans	Tsetse fly	Unassigned	Venom or Salivary	Glossina Glo m 5	259	289742475	11
774	Glossina morsitans morsitans	Tsetse fly	Unassigned	Venom or Salivary	Glossina Glo m 5	222	289742483	11
775	Glossina morsitans morsitans	Tsetse fly	Unassigned	Venom or Salivary	Glossina Glo m 5	259	8927462	11
776	Glycine max	Soybean	Unassigned	Food Plant	Gly m 5 Glycine Beta-conglycinin	605	18536	7
777	Glycine max	Soybean	Unassigned	Food Plant	Gly m 5 Glycine Beta-conglycinin	218	169927	7
778	Glycine max	Soybean	Unassigned	Food Plant	Gly m 5 Glycine Beta-conglycinin	639	169929	7
779	Glycine max	Soybean	Unassigned	Food Plant	Gly m 5 Glycine Beta-conglycinin	439	256427	7
780	Glycine max	Soybean	Gly m 1.0101	Aero Plant	Glycine Gly m 1	42	999355	7
781	Glycine max	Soybean	Unassigned	Food Plant	Glycine Gly m 1	134	76782247	7
782	Glycine max	Soybean	Unassigned	Food Plant	Glycine Gly m 1	119	76782249	7
783	Glycine max	Soybean	Gly m 2	Aero Plant	Glycine Gly m 2	20	1362049	7
784	Glycine max	Soybean	Gly m 3	Food Plant	Glycine Gly m 3	131	3021373	7
785	Glycine max	Soybean	Gly m 3	Food Plant	Glycine Gly m 3	131	3914435	7
786	Glycine max	Soybean	Unassigned	Food Plant	Glycine Gly m 3	131	156938901	9
787	Glycine max	Soybean	Unassigned	Food Plant	Glycine Gly m 4	158	134194	9
788	Glycine max	Soybean	Unassigned	Food Plant	Glycine Gly m Bd 28K	473	12697782	7
789	Glycine max	Soybean	Unassigned	Food Plant	Glycine Gly m Bd 28K	373	187766751	10

790	Glycine max	Soybean	Unassigned	Food Plant	Glycine Gly m Bd 28K	373	187766749	10
791	Glycine max	Soybean	Unassigned	Food Plant	Glycine Gly m Bd 28K	373	187766747	10
792	Glycine max	Soybean	Unassigned	Food Plant	Glycine Gly m Bd 28K	455	187766755	10
793	Glycine max	Soybean	Unassigned	Food Plant	Glycine Gly m Bd 30 kDa	379	129353	17
794	Glycine max	Soybean	Unassigned	Food Plant	Glycine Gly m Bd 30 kDa	379	1199563	17
795	Glycine max	Soybean	Unassigned	Food Plant	Glycine Gly m Bd 30 kDa	379	3097321	17
796	Glycine max	Soybean	Unassigned	Food Plant	Glycine Glycinin G1	495	18615	17
797	Glycine max	Soybean	Unassigned	Food Plant	Glycine Glycinin G1	495	18635	17
798	Glycine max	Soybean	Unassigned	Food Plant	Glycine Glycinin G2	485	18609	17
799	Glycine max	Soybean	Unassigned	Food Plant	Glycine Glycinin G2	485	18637	17
800	Glycine max	Soybean	Unassigned	Food Plant	Glycine Glycinin G3	481	18639	17
801	Glycine max	Soybean	Unassigned	Food Plant	Glycine Glycinin G4	562	18641	17
802	Glycine max	Soybean	Unassigned	Food Plant	Glycine Glycinin G4	562	732706	17
803	Glycine soja	Soybean	Unassigned	Food Plant	Glycine Glycinin G4	563	806556	17
804	Glycine max	Soybean	Unassigned	Food Plant	Glycine Glycinin G5	516	169969	17
805	Glycine max	Soybean	Unassigned	Food Plant	Glycine Glycinin G5	240	169971	17
806	Glycine soja	Soybean	Unassigned	Food Plant	Glycine Glycinin G5	517	736002	17
807	Glycine max	Soybean	Unassigned	Food Plant	Glycine Major Gly 50 kDa allergen	17	85681057	17
808	Glycine max	Soybean	Unassigned	Food Plant	Glycine Trypsin inhibitor	217	18770	17
809	Glycine max	Soybean	Unassigned	Food Plant	Glycine Trypsin inhibitor	217	18772	17
810	Glycine max	Soybean	Unassigned	Food Plant	Glycine Trypsin inhibitor	216	256429	17
811	Glycine max	Soybean	Unassigned	Food Plant	Glycine Trypsin inhibitor	203	256635	17
812	Glycine max	Soybean	Unassigned	Food Plant	Glycine Trypsin inhibitor	204	256636	17
813	Glycine max	Soybean	Unassigned	Food Plant	Glycine Trypsin inhibitor	208	510515	17
814	Glycyphagus domesticus	Storage mite	Unassigned	Aero Mite	Glycyphagus Gly d 2	141	33772588	17
815	Glycyphagus domesticus	Storage mite	Unassigned	Aero Mite	Glycyphagus Gly d 2	125	48428170	19
816	Glycyphagus	Storage mite	Unassigned	Aero Mite	Glycyphagus Gly d 2	128	48428178	19

	domesticus							
817	Haliotis discus discus	Disk abalone	Unassigned	Food Animal	Haliotis Hal m 1 tropomyosin	284	219806586	10
818	Haliotis diversicolor	Abalone	Unassigned	Food Animal	Haliotis Hal m 1 tropomyosin	284	9954249	7
819	Haliotis discus discus	Disk abalone	Unassigned	Food Animal	Haliotis paramyosin	860	318609972	12
820	Helianthus annuus	Sunflower	Hel a 2	Aero Plant	Helianthus Hel a 2	133	3581965	7
821	Helianthus annuus	Sunflower	Unassigned	Food Plant	Helianthus Seed 2S albumin	141	112745	9
822	Helix aspersa	Brown garden snail	Unassigned	Food Animal	Helix Hel as 1 tropomyosin	284	42559558	9
823	Hevea brasiliensis	Para rubber tree	Hev b 1	Contact	Hevea Hev b 1	138	132270	7
824	Hevea brasiliensis	Para rubber tree	Hev b 10.0101	Contact	Hevea Hev b 10	233	348137	7
825	Hevea brasiliensis	Para rubber tree	Hev b 10.0102	Contact	Hevea Hev b 10	205	5777414	7
826	Hevea brasiliensis	Para rubber tree	Hev b 10.0103	Contact	Hevea Hev b 10	205	10862818	7
827	Hevea brasiliensis	Para rubber tree	Hev b 11	Contact	Hevea Hev b 11	295	14575525	7
828	Hevea brasiliensis subsp. brasiliensis	Para rubber tree	Hev b 11	Contact	Hevea Hev b 11	295	27526732	7
829	Hevea brasiliensis	Para rubber tree	Hev b 12	Contact	Hevea Hev b 12	116	20135538	7
830	Hevea brasiliensis	Para rubber tree	Unassigned	Contact	Hevea Hev b 13	391	51315784	9
831	Hevea brasiliensis	Para rubber tree	Unassigned	Contact	Hevea Hev b 14 hevamine	208	313870530	12
832	Hevea brasiliensis	Para rubber tree	Hev b 2	Contact	Hevea Hev b 2	374	1184668	7
833	Hevea brasiliensis	Para rubber tree	Hev b 2	Contact	Hevea Hev b 2	374	32765543	7
834	Hevea brasiliensis	Para rubber tree	Unassigned	Contact	Hevea Hev b 2	374	124294783	8
835	Hevea brasiliensis	Para rubber tree	Unassigned	Contact	Hevea Hev b 2	374	124294785	8
836	Hevea brasiliensis	Para rubber tree	Unassigned	Contact	Hevea Hev b 2	374	124365249	8
837	Hevea brasiliensis	Para rubber tree	Unassigned	Contact	Hevea Hev b 2	374	124365251	8
838	Hevea brasiliensis	Para rubber tree	Unassigned	Contact	Hevea Hev b 2	374	124365253	8

839	Hevea brasiliensis	Para rubber tree	Unassigned	Contact	Hevea Hev b 2	316	261824817	11
840	Hevea brasiliensis	Para rubber tree	Unassigned	Contact	Hevea Hev b 2	374	268037674	11
841	Hevea brasiliensis	Para rubber tree	Unassigned	Contact	Hevea Hev b 2	374	270315180	11
842	Hevea brasiliensis	Para rubber tree	Hev b 3	Contact	Hevea Hev b 3	204	14423933	17
843	Hevea brasiliensis	Para rubber tree	Unassigned	Contact	Hevea Hev b 4	366	46410859	17
844	Hevea brasiliensis	Para rubber tree	Unassigned	Contact	Hevea Hev b 5	151	7387766	18
845	Hevea brasiliensis	Para rubber tree	Hev b 6	Contact	Hevea Hev b 6	204	123062	17
846	Hevea brasiliensis	Para rubber tree	Hev b 6	Contact	Hevea Hev b 6	187	2832430	17
847	Hevea brasiliensis	Para rubber tree	Unassigned	Contact	Hevea Hev b 6	43	73535415	17
848	Hevea brasiliensis	Para rubber tree	Unassigned	Contact	Hevea Hev b 6	204	158342650	19
849	Hevea brasiliensis	Para rubber tree	Hev b 7.01	Contact	Hevea Hev b 7	388	1916805	17
850	Hevea brasiliensis	Para rubber tree	Hev b 7.02	Contact	Hevea Hev b 7	388	3087805	17
851	Hevea brasiliensis	Para rubber tree	Unassigned	Contact	Hevea Hev b 7	388	3288200	17
852	Hevea brasiliensis	Para rubber tree	Hev b 7	Contact	Hevea Hev b 7	388	6707018	17
853	Hevea brasiliensis	Para rubber tree	Unassigned	Contact	Hevea Hev b 7	387	41581137	17
854	Hevea brasiliensis	Para rubber tree	Hev b 8	Contact	Hevea Hev b 8	131	3183706	17
855	Hevea brasiliensis	Para rubber tree	Hev b 8	Contact	Hevea Hev b 8	131	11513601	17
856	Hevea brasiliensis	Para rubber tree	Hev b 8.0204	Contact	Hevea Hev b 8	131	14423856	17
857	Hevea brasiliensis	Para rubber tree	Hev b 8.0203	Contact	Hevea Hev b 8	131	14423858	17
858	Hevea brasiliensis	Para rubber tree	Hev b 8.0202	Contact	Hevea Hev b 8	131	14423859	17
859	Hevea brasiliensis	Para rubber tree	Hev b 8.0201	Contact	Hevea Hev b 8	131	14423860	17
860	Hevea brasiliensis	Para rubber tree	Hev b 8.0102	Contact	Hevea Hev b 8	131	14423868	17
861	Hevea brasiliensis	Para rubber tree	Unassigned	Contact	Hevea Hev b 9	445	14423687	19

862	Hevea brasiliensis	Para rubber tree	Unassigned	Contact	Hevea Hev b 9	445	14423688	9
863	Holcus lanatus	Velvet grass	Unassigned	Aero Plant	Holcus group V	240	2266623	17
864	Holcus lanatus	Velvet grass	Unassigned	Aero Plant	Holcus group V	264	2266625	17
865	Holcus lanatus	Velvet grass	Unassigned	Aero Plant	Holcus group V	296	11991229	17
866	Holcus lanatus	Velvet grass	Hol 1 1.0102	Aero Plant	Holcus Hol 1 1	248	1167836	17
867	Holcus lanatus	Velvet grass	Unassigned	Aero Plant	Holcus Hol 1 1	263	3860384	17
868	Holcus lanatus	Velvet grass	Unassigned	Aero Plant	Holcus Hol 1 1	265	1171005	19
869	Homarus americanus	American lobster	Unassigned	Food Animal	Homarus Tropomyosin	284	2660868	17
870	Homarus americanus	American lobster	Unassigned	Food Animal	Homarus Tropomyosin	284	14285796	17
871	Hordeum vulgare subsp. vulgare	Barley	Unassigned	Aero Plant	Hordeum Alpha-amylase inhibitor BDAI-1	152	3367714	17
872	Hordeum vulgare subsp. vulgare	Barley	Unassigned	Aero Plant	Hordeum Alpha-amylase inhibitor component Cma	144	18955	17
873	Hordeum vulgare subsp. vulgare	Barley	Unassigned	Aero Plant	Hordeum Alpha-amylase inhibitor component Cma	145	439275	17
874	Hordeum vulgare	Barley	Unassigned	Aero Plant	Hordeum Alpha-amylase inhibitor component CMb	149	585290	17
875	Hordeum vulgare	Barley	Hor v 15	Aero Plant	Hordeum Hor v 15	146	2506771	17
876	Hordeum vulgare	Barley	Unassigned	Aero Plant	Hordeum LTP 1	117	167077	17
877	Hordeum vulgare	Barley	Unassigned	Food Plant	Hordeum LTP 1	134	19039	17
878	Hordeum vulgare	Barley	Unassigned	Aero Plant	Hordeum Trypsin inhibitor CMe	144	1405736	17
879	Hordeum vulgare subsp. vulgare	Barley	Unassigned	Aero Plant	Hordeum Trypsin inhibitor CMe	148	19009	17
880	Humulus japonicus	Japanese hop	Hum j 1	Aero Plant	Humulus Humj1	155	33113263	17
881	Humulus scandens	Japanese hop	Unassigned	Aero Plant	Humulus profilin-like protein	131	34851176	17
882	Humulus scandens	Japanese hop	Unassigned	Aero Plant	Humulus profilin-like protein	131	34851174	17
883	Juglans nigra	Black walnut	Jug n 1	Food Plant	Juglans Jug r 1	161	31321942	17
884	Juglans regia	English walnut	Jug r 1	Food Plant	Juglans Jug r 1	139	1794252	17

885	Juglans nigra	Black walnut	Jug n 2	Food Plant	Juglans Jug r 2	481	31321944	7
886	Juglans regia	English walnut	Jug r 2	Food Plant	Juglans Jug r 2	593	6580762	7
887	Juglans regia	English walnut	Unassigned	Food Plant	Juglans Jug r 3	119	209484145	11
888	Juglans regia	English walnut	Unassigned	Food Plant	Juglans Jug r 4 seed storage protein	507	56788031	7
889	Juniperus ashei	Mountain cedar	Unassigned	Aero Plant	Juniperus Jun a 2	507	47606048	9
890	Juniperus ashei	Mountain cedar	Unassigned	Aero Plant	Juniperus Jun a 3	225	9087177	8
891	Juniperus rigida	Cedar	Unassigned	Aero Plant	Juniperus Jun a 3	225	38456224	7
892	Juniperus rigida	Cedar	Unassigned	Aero Plant	Juniperus Jun a 3	225	38456222	7
893	Juniperus virginiana	Red cedar	Unassigned	Aero Plant	Juniperus Jun a 3	110	51316532	7
894	Juniperus ashei	Mountain cedar	Unassigned	Aero Plant	Juniperus Jun a/v 1	367	9087152	9
895	Juniperus oxycedrus	Juniper	Unassigned	Aero Plant	Juniperus Jun a/v 1	367	15139849	7
896	Juniperus virginiana	Red cedar	Jun v 1	Aero Plant	Juniperus Jun a/v 1	367	8843917	7
897	Juniperus virginiana	Red cedar	Jun v 1	Aero Plant	Juniperus Jun a/v 1	367	8843921	7
898	Juniperus oxycedrus	Juniper	Unassigned	Aero Plant	Juniperus Jun o 4	165	14423843	8
899	Lens culinaris	Lentil	Len c 1.0101	Food Plant	Lens Len c 1	418	29539109	7
900	Lens culinaris	Lentil	Len c 1.0102	Food Plant	Lens Len c 1	415	29539111	7
901	Lepidoglyphus destructor	Storage mite	Lep d 10	Aero Mite	Lepidoglyphus Lep d 10	284	14423956	7
902	Lepidoglyphus destructor	Storage mite	Lep d 13	Aero Mite	Lepidoglyphus Lep d 13	131	14423714	7
903	Lepidoglyphus destructor	Storage mite	Lep d 2	Aero Mite	Lepidoglyphus Lep d 2	141	2147108	7
904	Lepidoglyphus destructor	Storage mite	Lep d 2	Aero Mite	Lepidoglyphus Lep d 2	141	21213898	7
905	Lepidoglyphus destructor	Storage mite	Lep d 2	Aero Mite	Lepidoglyphus Lep d 2	141	21213900	7
906	Lepidoglyphus destructor	Storage mite	Lep d 2	Aero Mite	Lepidoglyphus Lep d 2	141	1582223	7
907	Lepidoglyphus destructor	Storage mite	Lep d 2	Aero Mite	Lepidoglyphus Lep d 2	141	1582222	7
908	Lepidoglyphus	Storage mite	Unassigned	Aero Mite	Lepidoglyphus Lep d 141	141	34495274	7

	destructor				2			
909	Lepidoglyphus destructor	Storage mite	Unassigned	Aero Mite	Lepidoglyphus Lep d 2	141	34495278	7
910	Lepidoglyphus destructor	Storage mite	Unassigned	Aero Mite	Lepidoglyphus Lep d 2	140	34495280	7
911	Lepidoglyphus destructor	Storage mite	Unassigned	Aero Mite	Lepidoglyphus Lep d 2	141	34495282	7
912	Lepidoglyphus destructor	Storage mite	Unassigned	Aero Mite	Lepidoglyphus Lep d 2	141	34495284	7
913	Lepidoglyphus destructor	Storage mite	Unassigned	Aero Mite	Lepidoglyphus Lep d 2	141	34495286	7
914	Lepidoglyphus destructor	Storage mite	Unassigned	Aero Mite	Lepidoglyphus Lep d 2	141	34495288	7
915	Lepidoglyphus destructor	Storage mite	Unassigned	Aero Mite	Lepidoglyphus Lep d 2	141	34495290	7
916	Lepidoglyphus destructor	Storage mite	Unassigned	Aero Mite	Lepidoglyphus Lep d 2	141	1708793	9
917	Lepidoglyphus destructor	Storage mite	Lep d 5	Aero Mite	Lepidoglyphus Lep d 5	110	14423651	7
918	Lepidoglyphus destructor	Storage mite	Unassigned	Aero Mite	Lepidoglyphus Lep d 5	171	34495292	7
919	Lepidoglyphus destructor	Storage mite	Unassigned	Aero Mite	Lepidoglyphus Lep d 5	169	34495294	7
920	Lepidoglyphus destructor	Storage mite	Lep d 7	Aero Mite	Lepidoglyphus Lep d 7	216	14423650	7
921	Lepidorhombus whiffiagonis		Unassigned	Food Animal	Lepidorhombus Lep w 1 parvalbumin	109	208608078	10
922	Lepisma saccharina	Silverfish	Lep s 1	Aero Insect	Lepisma Tropomyosin	284	20387027	7
923	Lepisma saccharina	Silverfish	Unassigned	Aero Insect	Lepisma Tropomyosin	243	20387029	7
924	Ligustrum vulgare	Privet	Lig v 1.0102	Aero Plant	Ligustrum Lig v 1	145	3256212	7
925	Ligustrum vulgare	Privet	Unassigned	Aero Plant	Ligustrum Lig v 1	145	14423737	8
926	Lilium longiflorum	Trumpet lily	Unassigned	Aero Plant	Lilium polygalacturonase	413	73913442	8
927	Litchi chinensis	Lychee nut	Lit c 1	Food Plant	Litchi Lit c 1	131	15809696	7
928	Litchi chinensis	Lychee nut	Unassigned	Food Plant	Litchi Lit c 1	131	83317152	7
929	Litopenaeus vannamei		Unassigned	Food Animal	Litopenaeus Lit v 4 sarcoplasmic Ca+ binding	193	223403273	11
930	Litopenaeus vannamei		Unassigned	Food Animal	Litopenaeus Lit v 2	356	115492980	8
931	Litopenaeus vannamei		Unassigned	Food Animal	Litopenaeus Lit v 3 myosin	177	184198734	10

932	Lolium perenne ryegrass	Perennial	Lol p 1	Aero Plant	Lolium Lol p 1	263	126385	7
933	Lolium perenne ryegrass	Perennial	Lol p 1	Aero Plant	Lolium Lol p 1	252	168314	7
934	Lolium perenne ryegrass	Perennial	Lol p 1	Aero Plant	Lolium Lol p 1	263	75274600	7
935	Lolium perenne ryegrass	Perennial	Unassigned	Aero Plant	Lolium Lol p 1	263	168316	10
936	Lolium perenne ryegrass	Perennial	Lol p 11	Aero Plant	Lolium Lol p 11	134	47605808	7
937	Lolium perenne ryegrass	Perennial	Lol p 2	Aero Plant	Lolium Lol p 2	97	126386	7
938	Lolium perenne ryegrass	Perennial	Lol p 2	Aero Plant	Lolium Lol p 2	88	939932	7
939	Lolium perenne ryegrass	Perennial	Lol p 3	Aero Plant	Lolium Lol p 3	97	126387	7
940	Lolium perenne ryegrass	Perennial	Unassigned	Aero Plant	Lolium Lol p 4	423	55859464	7
941	Lolium perenne ryegrass	Perennial	Lol p 5.0101	Aero Plant	Lolium Lol p 5 *ver 10	339	2498582	7
942	Lolium perenne ryegrass	Perennial	Lol p 5	Aero Plant	Lolium Lol p 5 *ver 10	301	4416516	7
943	Lolium perenne ryegrass	Perennial	Lol p 5	Aero Plant	Lolium Lol p 5 *ver 10	301	6634467	7
944	Lolium perenne ryegrass	Perennial	Unassigned	Aero Plant	Lolium Lol p 5 *ver 10	307	332278195	12
945	Lupinus angustifolius		Unassigned	Food Plant	Lupinus conglutin beta	521	149208401	9
946	Lupinus angustifolius		Unassigned	Food Plant	Lupinus conglutin beta	455	149208403	9
947	Lupinus angustifolius		Unassigned	Food Plant	Lupinus conglutin beta	611	169950562	10
948	Lycopersicon esculentum	Tomato	Lyc e 1	Food Plant	Lycopersicon Lyc e 1	131	16555787	7
949	Lycopersicon esculentum	Tomato	Lyc e 1	Food Plant	Lycopersicon Lyc e 1	131	17224229	7
950	Lycopersicon esculentum	Tomato	Lyc e 2.0101	Food Plant	Lycopersicon Lyc e 2	553	18542113	7
951	Lycopersicon esculentum	Tomato	Lyc e 2.0102	Food Plant	Lycopersicon Lyc e 2	636	18542115	7
952	Lycopersicon esculentum	Tomato	Unassigned	Food Plant	Lycopersicon Lyc e 3	114	71360928	7
953	Lycopersicon esculentum	Tomato	Unassigned	Food Plant	Lycopersicon Lyc e 3	114	71360930	7
954	Macrobrachium rosenbergii		Unassigned	Animal	Macrobrachium rosenbergii shrimp	284	288819271	11

					tropomyosin			
955	Malassezia furfur	Yeast	Unassigned	Contact	Malassezia Mala f 2	177	3914386	8
956	Malassezia furfur	Yeast	Unassigned	Contact	Malassezia Mala f 3	166	3914387	8
957	Malassezia furfur	Yeast	Mala f 4	Contact	Malassezia Mala f 4	342	4587985	7
958	Malassezia furfur	Yeast	Unassigned	Contact	Malassezia Mala s 1	350	13959403	7
959	Malassezia sympodialis	Yeast	Mala s 11	Contact	Malassezia Mala s 11	237	28569698	7
960	Malassezia sympodialis	Yeast	Unassigned	Contact	Malassezia Mala s 12	618	78038796	7
961	Malassezia sympodialis	Yeast	Unassigned	Contact	Malassezia Mala s 13 Thioredoxin Rev	121	119390336	8
962	Malassezia sympodialis	Yeast	Mala s 5	Contact	Malassezia Mala s 5	172	4138171	7
963	Malassezia sympodialis	Yeast	Mala s 6	Contact	Malassezia Mala s 6	162	4138173	7
964	Malassezia sympodialis	Yeast	Mala s 7	Contact	Malassezia Mala s 7	187	4138175	7
965	Malassezia sympodialis	Yeast	Mala s 8	Contact	Malassezia Mala s 8	179	7271239	7
966	Malassezia sympodialis	Yeast	Mala s 9	Contact	Malassezia Mala s 9	342	19069920	7
967	Malus x domestica	Apple	Mal d 1	Food Plant	Malus Mal d 1	159	1313966	7
968	Malus x domestica	Apple	Mal d 1	Food Plant	Malus Mal d 1	159	4590364	7
969	Malus x domestica	Apple	Mal d 1	Food Plant	Malus Mal d 1	159	4590366	7
970	Malus x domestica	Apple	Mal d 1	Food Plant	Malus Mal d 1	159	4590368	7
971	Malus x domestica	Apple	Mal d 1	Food Plant	Malus Mal d 1	159	4590376	7
972	Malus x domestica	Apple	Mal d 1	Food Plant	Malus Mal d 1	159	4590378	7
973	Malus x domestica	Apple	Mal d 1	Food Plant	Malus Mal d 1	159	4590380	7
974	Malus x domestica	Apple	Mal d 1	Food Plant	Malus Mal d 1	159	4590382	7
975	Malus x domestica	Apple	Mal d 1	Food Plant	Malus Mal d 1	159	4590388	7
976	Malus x domestica	Apple	Mal d 1	Food Plant	Malus Mal d 1	159	16555783	7
977	Malus x	Apple	Mal d 1	Food Plant	Malus Mal d 1	159	27922941	7

	domestica							
978	Malus x domestica	Apple	Mal d 1	Food Plant	Malus Mal d 1	159	1346478	7
979	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 1	159	60280829	7
980	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 1	159	60280851	7
981	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 1	159	42558971	9
982	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 1	159	75306008	11
983	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 1	159	75306007	11
984	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 1	159	886683	11
985	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 2	26	1478293	7
986	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 2	246	60418842	7
987	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 2	246	60418848	7
988	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 2	246	30316292	8
989	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 2	158	218059718	10
990	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 2	158	218059715	10
991	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 3	115	50659891	7
992	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 3	115	50659889	7
993	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 3	115	50659885	7
994	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 3	115	50659879	7
995	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 3	115	50659859	7
996	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 3	115	38492338	7
997	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 3	115	14423814	9
998	Malus x domestica	Apple	Mal d 4	Food Plant	Malus Mal d 4	131	14423873	7
999	Malus x domestica	Apple	Mal d 4	Food Plant	Malus Mal d 4	131	14423874	7
1000	Malus x domestica	Apple	Mal d 4	Food Plant	Malus Mal d 4	131	14423875	7

	domestica							
1001	Malus x domestica	Apple	Mal d 4	Food Plant	Malus Mal d 4	131	28881453	7
1002	Malus x domestica	Apple	Mal d 4	Food Plant	Malus Mal d 4	131	28881457	7
1003	Malus x domestica	Apple	Mal d 4	Food Plant	Malus Mal d 4	131	28881455	7
1004	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 4	131	60418854	7
1005	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 4	131	60418858	7
1006	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 4	131	60418862	7
1007	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 4	131	60418866	7
1008	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 4	131	164510842	9
1009	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 4	131	164510858	9
1010	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 4	131	164510860	9
1011	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 4	77	218059730	10
1012	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 4	115	218059733	10
1013	Malus x domestica	Apple	Unassigned	Food Plant	Malus Mal d 4	131	218059728	10
1014	Marsupenaeus japonicus		Unassigned	Food Animal	Marsupenaeus tropomyosin	284	125995159	8
1015	Mercurialis annua	Annual mercury grass	Mer a 1	Aero Plant	Mercurialis Mer a 1	133	2959898	7
1016	Metapenaeus ensis	Greasyback shrimp	Unassigned	Food Animal	Metapenaeus Met e 1 Tropomyosin	274	6094504	9
1017	Mimachlamys nobilis	Noble scallop	Unassigned	Food Animal	Mimachlamys Tropomyosin	284	9954253	7
1018	Morus nigra	Black mulberry	Unassigned	Food Plant	Morus Mor n 3 mulberry LTP	91	288561913	11
1019	Mus musculus	Mouse	Mus m 1	Aero Animal	Mus Mus m 1	180	20178291	7
1020	Musa acuminata	Banana	Mus xp 1	Food Plant	Musa profilin banana	131	14161635	7
1021	Myrmecia pilosula	Jumper ant	Myr p 1	Venom or Salivary	Myrmecia Myr p 1	112	730091	7
1022	Myrmecia pilosula	Jumper ant	Unassigned	Venom or Salivary	Myrmecia Myr p 1	112	1911819	7
1023	Myrmecia	Jumper ant	Myr p 2	Venom or	Myrmecia Myr p 2	75	1587177	7

	pilosula			Salivary				
1024	Myrmecia pilosula	Jumper ant	Myr p 2	Venom or Salivary	Myrmecia Myr p 2	75	2498604	7
1025	Neptunea polycostata		Unassigned	Food Animal	Neptunea tropomyosin	284	219806590	10
1026	Nicotiana tabacum	Tobacco	Unassigned	Aero Plant	Nicotiana villin	520	57283139	7
1027	Nicotiana tabacum	Tobacco	Unassigned	Aero Plant	Nicotiana villin	559	57283137	7
1028	Octopus vulgaris		Unassigned	Food Animal	Octopus tropomyosin	284	83715936	7
1029	Olea europaea	Olive tree	Ole e 1	Aero Plant	Olea Ole e 1	145	14424429	7
1030	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 1	137	1362128	7
1031	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 1	136	1362129	7
1032	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 1	136	1362130	7
1033	Olea europaea	Olive tree	Ole e 1.0104	Aero Plant	Olea Ole e 1	145	1362131	7
1034	Olea europaea	Olive tree	Ole e 1	Aero Plant	Olea Ole e 1	137	1362132	7
1035	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 1	136	1362133	7
1036	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 1	136	1362134	7
1037	Olea europaea	Olive tree	Ole e 1.0102	Aero Plant	Olea Ole e 1	145	1362135	7
1038	Olea europaea	Olive tree	Ole e 1.0103	Aero Plant	Olea Ole e 1	145	1362136	7
1039	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 1	136	1362137	7
1040	Olea europaea	Olive tree	Ole e 1.0105	Aero Plant	Olea Ole e 1	146	2465127	7
1041	Olea europaea	Olive tree	Ole e 1.0106	Aero Plant	Olea Ole e 1	146	2465129	7
1042	Olea europaea	Olive tree	Ole e 1.0107	Aero Plant	Olea Ole e 1	146	2465131	7
1043	Olea europaea	Olive tree	Ole e 1.0101	Aero Plant	Olea Ole e 1	130	13195753	7
1044	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 1	134	37724597	7
1045	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 1	135	37724593	7
1046	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 1	132	37548753	7
1047	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 1	131	33329758	7
1048	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 1	132	33329756	7
1049	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 1	132	33329754	7
1050	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 1	131	33329752	7
1051	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 1	131	33329750	7

1052	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 1	129	33329748	7
1053	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 1	131	33329744	7
1054	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 1	132	33329738	7
1055	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 1	132	33329732	7
1056	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 1	132	33325115	7
1057	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 1	140	145313982	9
1058	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 1	140	145313984	9
1059	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 1	140	145313988	9
1060	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 1	140	145313990	9
1061	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 1	140	145313992	9
1062	Olea europaea	Olive tree	Ole e 10	Aero Plant	Olea Ole e 10	123	29465664	7
1063	Olea europaea	Olive tree	Ole e 11.0102	Aero Plant	Olea Ole e 11.0101 and 0102	364	68270856	11
1064	Olea europaea	Olive tree	Ole e 11.0101	Aero Plant	Olea Ole e 11.0101 and 0102	364	269996495	11
1065	Olea europaea	Olive tree	Unassigned	Food Plant	Olea Ole e 13 thaumatin	226	269996497	12
1066	Olea europaea	Olive tree	Ole e 2	Aero Plant	Olea Ole e 2	134	3914426	7
1067	Olea europaea	Olive tree	Ole e 2	Aero Plant	Olea Ole e 2	134	3914427	7
1068	Olea europaea	Olive tree	Ole e 2	Aero Plant	Olea Ole e 2	134	3914428	7
1069	Olea europaea	Olive tree	Ole e 3	Aero Plant	Olea Ole e 3	84	3337403	7
1070	Olea europaea	Olive tree	Ole e 3	Aero Plant	Olea Ole e 3	52	37725377	7
1071	Olea europaea	Olive tree	Ole e 5	Aero Plant	Olea Ole e 5	30	122064581	8
1072	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 5	152	145313972	9
1073	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 5	152	160347106	9
1074	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 5	144	160347108	9
1075	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 5	152	160347112	9
1076	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 5	152	160347120	9
1077	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 5	152	160347122	9
1078	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 5	152	160347124	9
1079	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 5	152	160347126	9
1080	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 5	152	160347130	9
1081	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 5	152	160347134	9
1082	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 5	152	160347138	9
1083	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 5	152	160962543	9
1084	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 5	152	160962547	9

1085	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 5	152	160962557	9
1086	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 5	152	160962577	9
1087	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 5	152	160962583	9
1088	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 5	144	160962587	9
1089	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 5	152	160962591	9
1090	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 5	152	160962597	9
1091	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 5	152	160962611	9
1092	Olea europaea	Olive tree	Ole e 6	Aero Plant	Olea Ole e 6	50	14423643	7
1093	Olea europaea	Olive tree	Ole e 7	Aero Plant	Olea Ole e 7	21	22002032	7
1094	Olea europaea	Olive tree	Ole e 8	Aero Plant	Olea Ole e 8	171	6901654	7
1095	Olea europaea	Olive tree	Ole e 8	Aero Plant	Olea Ole e 8	171	14423648	7
1096	Olea europaea	Olive tree	Ole e 9	Aero Plant	Olea Ole e 9	460	14279169	7
1097	Olea europaea	Olive tree	Unassigned	Aero Plant	Olea Ole e 9	101	166235350	9
1098	Ommastrephes bartramii		Unassigned	Food Animal	Ommastrephes tropomyosin	284	83715934	7
1099	Onchocerca volvulus	Parasitic nematode	Unassigned	Worm (parasite)	Onchocerca tropomyosin	284	42559586	12
1100	Oncorhynchus mykiss		Unassigned	Food Animal	Oncorhynchus Rainbow trout parv Onc m 1	108	288559139	11
1101	Oncorhynchus mykiss		Unassigned	Food Animal	Oncorhynchus Rainbow trout parv Onc m 1	107	288559140	11
1102	Oratosquilla oratoria		Unassigned	Food Animal	Oratosquilla tropomyosin	284	162286975	9
1103	Oryza sativa	Rice	Unassigned	Food Plant	Oryza Glyoxalase I	291	84029333	7
1104	Oryza sativa (japonica cultivar-group)	Rice	Unassigned	Food Plant	Oryza Glyoxalase I	291	16580747	7
1105	Oryza sativa	Rice	Ory s 1	Aero Plant	Oryza Ory s 1	263	1173557	8
1106	Oryza sativa	Rice	Unassigned	Aero Plant	Oryza Ory s 1	267	8118439	7
1107	Oryza sativa (japonica cultivar-group)	Rice	Ory s 1	Aero Plant	Oryza Ory s 1	267	109913547	8
1108	Oryza sativa (japonica cultivar-group)	Rice	Unassigned	Food Plant	Oryza Trypsin alpha-amylase inhibitor UNCERTAIN	157	23616954	8
1109	Oryza sativa (japonica cultivar-group)	Rice	Unassigned	Food Plant	Oryza Trypsin alpha-amylase inhibitor UNCERTAIN	165	218193	7
1110	Oryza sativa (japonica cultivar-group)	Rice	Unassigned	Food Plant	Oryza Trypsin alpha-amylase inhibitor UNCERTAIN	157	218197	7

1111	Oryza sativa (japonica cultivar-group)	Rice	Unassigned	Food Plant	Oryza Trypsin alpha-amylase inhibitor UNCERTAIN	111	1304216	7
1112	Oryza sativa (japonica cultivar-group)	Rice	Unassigned	Food Plant	Oryza Trypsin alpha-amylase inhibitor UNCERTAIN	109	1304217	7
1113	Oryza sativa (japonica cultivar-group)	Rice	Unassigned	Food Plant	Oryza Trypsin alpha-amylase inhibitor UNCERTAIN	113	1304218	7
1114	Oryza sativa (japonica cultivar-group)	Rice	Unassigned	Food Plant	Oryza Trypsin alpha-amylase inhibitor UNCERTAIN	166	1398913	7
1115	Oryza sativa (japonica cultivar-group)	Rice	Unassigned	Food Plant	Oryza Trypsin alpha-amylase inhibitor UNCERTAIN	160	1398915	7
1116	Oryza sativa (japonica cultivar-group)	Rice	Unassigned	Food Plant	Oryza Trypsin alpha-amylase inhibitor UNCERTAIN	157	1398916	7
1117	Oryza sativa (japonica cultivar-group)	Rice	Unassigned	Food Plant	Oryza Trypsin alpha-amylase inhibitor UNCERTAIN	160	1398918	7
1118	Oryza sativa (japonica cultivar-group)	Rice	Unassigned	Food Plant	Oryza Trypsin alpha-amylase inhibitor UNCERTAIN	157	2827316	7
1119	Oryza sativa (japonica cultivar-group)	Rice	Unassigned	Food Plant	Oryza Trypsin alpha-amylase inhibitor UNCERTAIN	166	114152865	8
1120	Oryza sativa (japonica cultivar-group)	Rice	Unassigned	Food Plant	Oryza Trypsin alpha-amylase inhibitor UNCERTAIN	163	114152864	8
1121	Oryza sativa (japonica cultivar-group)	Rice	Unassigned	Food Plant	Oryza Trypsin alpha-amylase inhibitor UNCERTAIN	160	23495787	8
1122	Oryza sativa (japonica cultivar-group)	Rice	Unassigned	Food Plant	Oryza Trypsin alpha-amylase inhibitor UNCERTAIN	160	23616947	7
1123	Ostrya carpinifolia		Unassigned	Aero Plant	Ostrya Ost c 1pollen allergen	160	300872535	12
1124	Pachycondyla chinensis		Unassigned	Venom or Salivary	Pachycondyla Pac c 3 allergen	199	169822894	10
1125	Pandalus borealis		Unassigned	Food Animal	Pandalus Pan b 1	284	312831088	12
1126	Pandalus eous		Unassigned	Food Animal	Pandalus Pan b 1	284	125995161	8
1127	Panulirus stimpsoni	Lobster	Unassigned	Food Animal	Panulirus Pan s 1	274	14285797	7
1128	Paralithodes camtschaticus		Unassigned	Food Animal	Paralithodes tropomyosin	284	125995163	8
1129	Paralithodes camtschaticus		Unassigned	Food Animal	Paralithodes tropomyosin	284	125995165	8

1130	Parietaria judaica	Weed	Par j 1	Aero Plant	Parietaria Par j 1	143	741844	17
1131	Parietaria judaica	Weed	Par j 1.0102	Aero Plant	Parietaria Par j 1	176	1532058	17
1132	Parietaria judaica	Weed	Par j 1.0201	Aero Plant	Parietaria Par j 1	138	2497749	17
1133	Parietaria judaica	Weed	Par j 1.0101	Aero Plant	Parietaria Par j 1	139	3915783	17
1134	Parietaria judaica	Weed	Par j 2.0102	Aero Plant	Parietaria Par j 2	133	1532056	17
1135	Parietaria judaica	Weed	Par j 2.0101	Aero Plant	Parietaria Par j 2	133	2497750	17
1136	Parietaria judaica	Weed	Par j 3	Aero Plant	Parietaria Par j 3	131	14423869	17
1137	Parietaria judaica	Weed	Par j 3	Aero Plant	Parietaria Par j 3	132	14423876	17
1138	Parietaria officinalis	Weed	Par o 1	Aero Plant	Parietaria Par o 1	12	75139847	17
1139	Parietaria officinalis	Weed	Par o 1	Aero Plant	Parietaria Par o 1	17	1311509	17
1140	Parietaria officinalis	Weed	Par o 1	Aero Plant	Parietaria Par o 1	15	1311510	17
1141	Parietaria officinalis	Weed	Par o 1	Aero Plant	Parietaria Par o 1	15	1311511	17
1142	Parietaria officinalis	Weed	Par o 1	Aero Plant	Parietaria Par o 1	15	1311512	17
1143	Parietaria officinalis	Weed	Par o 1	Aero Plant	Parietaria Par o 1	30	1311513	17
1144	Parietaria officinalis	Weed	Par o 1	Aero Plant	Parietaria Par o 1	24	1836011	17
1145	Parietaria officinalis	Weed	Unassigned	Aero Plant	Parietaria Par o 1	25	1836010	17
1146	Paspalum notatum	Bahia grass	Unassigned	Aero Plant	Paspalum group 13 pollen allergen	169	338930686	12
1147	Paspalum notatum	Bahia grass	Unassigned	Aero Plant	Paspalum group 13 pollen allergen	169	338930684	12
1148	Paspalum notatum	Bahia grass	Unassigned	Aero Plant	Paspalum group 13 pollen allergen	169	338930682	12
1149	Paspalum notatum	Bahia grass	Unassigned	Aero Plant	Paspalum group 13 pollen allergen	169	338930680	12
1150	Paspalum notatum	Bahia grass	Unassigned	Aero Plant	Paspalum group 13 pollen allergen	393	338930678	12
1151	Paspalum notatum	Bahia grass	Unassigned	Aero Plant	Paspalum group 13 pollen allergen	393	338930676	12
1152	Paspalum notatum	Bahia grass	Unassigned	Aero Plant	Paspalum group 13 pollen allergen	391	338930674	12

1153	Paspalum notatum	Bahia grass	Unassigned	Aero Plant	Paspalum group 13 pollen allergen	395	338930672	12
1154	Paspalum notatum	Bahia grass	Unassigned	Aero Plant	Paspalum Pas n 1 beta expansin	265	168419914	10
1155	Penaeus monodon	Black tiger shrimp	Unassigned	Food Animal	Penaeus Pen m 1 tropomyosin	284	125995157	8
1156	Penaeus monodon	Black tiger shrimp	Pen m 2	Food Animal	Penaeus Pen m 2	356	27463265	7
1157	Penaeus monodon	Black tiger shrimp	Unassigned	Food Animal	Penaeus Pen m 2	356	308154236	12
1158	Penaeus monodon	Black tiger shrimp	Unassigned	Food Animal	Penaeus Pen m 3 myosin light chain	177	317383196	12
1159	Penaeus monodon	Black tiger shrimp	Unassigned	Food Animal	Penaeus Pen m 4 sarcoplasmic calcium binding	193	317383198	12
1160	Penicillium chrysogenum	Fungus	Pen ch 18	Aero Fungi	Penicillium Pen 18	494	7963902	7
1161	Penicillium chrysogenum	Fungus	Pen ch 18	Aero Fungi	Penicillium Pen 18	494	14215732	7
1162	Penicillium citrinum	Fungus	Unassigned	Aero Fungi	Penicillium Pen 18	457	4588118	7
1163	Penicillium citrinum	Fungus	Unassigned	Aero Fungi	Penicillium Pen 18	358	12005501	7
1164	Penicillium oxalicum	Fungus	Pen o 18	Aero Fungi	Penicillium Pen 18	503	12005497	7
1165	Penicillium brevicompactum	Fungus	Unassigned	Aero Fungi	Penicillium Pen b 26	107	59894749	7
1166	Penicillium citrinum	Fungus	Pen c 19	Aero Fungi	Penicillium Pen c 19	503	14423733	7
1167	Penicillium citrinum	Fungus	Unassigned	Aero Fungi	Penicillium Pen c 22	438	74664773	9
1168	Penicillium citrinum	Fungus	Pen c 24	Aero Fungi	Penicillium Pen c 24	228	38326693	7
1169	Penicillium citrinum	Fungus	Pen c 3	Aero Fungi	Penicillium Pen c 3	167	5326864	7
1170	Penicillium chrysogenum	Fungus	Pen ch 13	Aero Fungi	Penicillium Pen ch 13	397	6684758	7
1171	Penicillium chrysogenum	Fungus	Pen ch 13	Aero Fungi	Penicillium Pen ch 13	398	21069093	7
1172	Penicillium citrinum	Fungus	Unassigned	Aero Fungi	Penicillium Pen ch 13	397	4587983	7
1173	Penicillium chrysogenum	Fungus	Pen ch 20	Aero Fungi	Penicillium Pen ch 20 68 kDa protein	117	999009	7
1174	Periplaneta americana	American cockroach	Per a 7.0101	Aero Insect	Periplaneta Per 7	284	4378573	7
1175	Periplaneta americana	American cockroach	Unassigned	Aero	Periplaneta Per 7	284	14423957	9

	americana	cockroach		Insect				
1176	Periplaneta americana	American cockroach	Unassigned	Aero Insect	Periplaneta Per a 7	284	239740599	11
1177	Periplaneta fuliginosa	Smokybrown cockroach	Unassigned	Aero Insect	Periplaneta Per a 7	284	19310971	7
1178	Periplaneta americana	American cockroach	Per a 1	Aero Insect	Periplaneta Per a 1	446	2231297	7
1179	Periplaneta americana	American cockroach	Per a 1.0104	Aero Insect	Periplaneta Per a 1	274	2253610	7
1180	Periplaneta americana	American cockroach	Per a 1	Aero Insect	Periplaneta Per a 1	395	2580504	7
1181	Periplaneta americana	American cockroach	Per a 1.0102	Aero Insect	Periplaneta Per a 1	228	2897849	7
1182	Periplaneta americana	American cockroach	Per a 1.0101	Aero Insect	Periplaneta Per a 1	231	4240399	7
1183	Periplaneta americana	American cockroach	Unassigned	Aero Insect	Periplaneta Per a 1	124	30144660	7
1184	Periplaneta americana	American cockroach	Unassigned	Aero Insect	Periplaneta Per a 1	395	284518361	11
1185	Periplaneta americana	American cockroach	Per a 3.0201	Aero Insect	Periplaneta Per a 3	631	1531589	7
1186	Periplaneta americana	American cockroach	Per a 3.0202	Aero Insect	Periplaneta Per a 3	470	1580794	7
1187	Periplaneta americana	American cockroach	Per a 3.0203	Aero Insect	Periplaneta Per a 3	393	1580797	7
1188	Periplaneta americana	American cockroach	Per a 3.0101	Aero Insect	Periplaneta Per a 3	685	2833325	9
1189	Periplaneta americana	American cockroach	Unassigned	Aero Insect	Periplaneta Per a 3	688	284518363	11
1190	Periplaneta americana	American cockroach	Unassigned	Aero Insect	Periplaneta Per a 3	685	289721058	11
1191	Periplaneta americana	American cockroach	Unassigned	Aero Insect	Periplaneta putative Per a 4	183	60678787	7
1192	Periplaneta americana	American cockroach	Unassigned	Aero Insect	Periplaneta putative Per a 4	163	215794707	10
1193	Periplaneta americana	American cockroach	Unassigned	Aero Insect	Periplaneta putative Per a 4	167	212675312	10
1194	Perna viridis	Asian green mussell	Unassigned	Food Animal	Perna Tropomyosin	284	9954251	7
1195	Persea americana	Avocado	Pers a 1	Food Plant	Persea Pers a 1	326	3201547	7
1196	Phalaris aquatica	Canary grass	Unassigned	Aero Plant	Phalaris Pha a 1	20	409328	7
1197	Phalaris aquatica	Canary grass	Pha a 1	Aero Plant	Phalaris Pha a 1	269	2498576	7
1198	Phalaris aquatica	Canary grass	Unassigned	Aero Plant	Phalaris Pha a 5	320	2498577	7

1199	Phalaris aquatica	Canary grass	Unassigned	Aero Plant	Phalaris Pha a 5	305	2498578	7
1200	Phalaris aquatica	Canary grass	Unassigned	Aero Plant	Phalaris Pha a 5	294	2498579	7
1201	Phalaris aquatica	Canary grass	Unassigned	Aero Plant	Phalaris Pha a 5	175	2498580	7
1202	Phaseolus vulgaris	Kidney bean	Unassigned	Food Plant	Phaseolus Pha v 3	115	289064177	11
1203	Phaseolus vulgaris	Kidney bean	Unassigned	Food Plant	Phaseolus Pha v 3	118	289064179	11
1204	Phleum pratense	Common timothy	Phl p 1.0101	Aero Plant	Phleum Phl p 1	263	3901094	7
1205	Phleum pratense	Common timothy	Phl p 1	Aero Plant	Phleum Phl p 1	241	28373838	7
1206	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 1	240	45823012	7
1207	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 1	263	1171008	9
1208	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 1	262	1582250	10
1209	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 11	143	47606039	9
1210	Phleum pratense	Common timothy	Phl p 12	Aero Plant	Phleum Phl p 12	131	464471	7
1211	Phleum pratense	Common timothy	Phl p 12	Aero Plant	Phleum Phl p 12	131	2415700	7
1212	Phleum pratense	Common timothy	Phl p 12	Aero Plant	Phleum Phl p 12	131	2415702	7
1213	Phleum pratense	Common timothy	Phl p 13	Aero Plant	Phleum Phl p 13	394	4826572	7
1214	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 2	122	1171009	8
1215	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 4	525	82492267	7
1216	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 4	508	54144332	7
1217	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 4	500	45108973	7
1218	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 4	500	45108967	7
1219	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 4	500	189014266	10
1220	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 4	500	189014268	10
1221	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 4	500	189014270	10

1222	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 4	500	189014272	10
1223	Phleum pratense	Common timothy	Phl p 5.0101	Aero Plant	Phleum Phl p 5	312	398830	17
1224	Phleum pratense	Common timothy	Phl p 5	Aero Plant	Phleum Phl p 5	257	422005	17
1225	Phleum pratense	Common timothy	Phl p 5	Aero Plant	Phleum Phl p 5	280	481397	17
1226	Phleum pratense	Common timothy	Phl p 5	Aero Plant	Phleum Phl p 5	24	75139900	17
1227	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 5	285	1092249	17
1228	Phleum pratense	Common timothy	Phl p 5.0202	Aero Plant	Phleum Phl p 5	281	1684718	17
1229	Phleum pratense	Common timothy	Phl p 5.0104	Aero Plant	Phleum Phl p 5	276	1684720	17
1230	Phleum pratense	Common timothy	Phl p 5	Aero Plant	Phleum Phl p 5	286	2398757	17
1231	Phleum pratense	Common timothy	Phl p 5	Aero Plant	Phleum Phl p 5	284	2851457	17
1232	Phleum pratense	Common timothy	Phl p 5.0105	Aero Plant	Phleum Phl p 5	276	3135497	17
1233	Phleum pratense	Common timothy	Phl p 5.0106	Aero Plant	Phleum Phl p 5	276	3135499	17
1234	Phleum pratense	Common timothy	Phl p 5.0107	Aero Plant	Phleum Phl p 5	276	3135501	17
1235	Phleum pratense	Common timothy	Phl p 5.0108	Aero Plant	Phleum Phl p 5	276	3135503	17
1236	Phleum pratense	Common timothy	Phl p 5.0103	Aero Plant	Phleum Phl p 5	312	3309039	17
1237	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 5	295	3309041	17
1238	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 5	290	3309045	17
1239	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 5	287	3309047	17
1240	Phleum pratense	Common timothy	Phl p 5	Aero Plant	Phleum Phl p 5	275	13430402	17
1241	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 5	287	21725606	17
1242	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 5	287	21725608	17
1243	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 5	287	21725610	17
1244	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 5	287	21725612	17

1245	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 5	287	21725614	17
1246	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 5	287	21725616	17
1247	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 5	287	21725618	17
1248	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 5	287	21725620	17
1249	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 5	287	21725622	17
1250	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 5	287	21725624	17
1251	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 5	287	21725626	17
1252	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 5	287	21725628	17
1253	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 5	287	21725630	17
1254	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 5	287	21725632	17
1255	Phleum pratense	Common timothy	Phl p 5	Aero Plant	Phleum Phl p 5	102	28948464	17
1256	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 5	284	29500897	17
1257	Phleum pratense	Common timothy	Phl p 6	Aero Plant	Phleum Phl p 6	138	3004465	17
1258	Phleum pratense	Common timothy	Phl p 6	Aero Plant	Phleum Phl p 6	138	3004467	17
1259	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 6	106	3004469	17
1260	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 6	111	28374072	17
1261	Phleum pratense	Common timothy	Unassigned	Aero Plant	Phleum Phl p 7	78	14423846	17
1262	Phoenix dactylifera	Date palm	Pho d 2	Aero Plant	Phoenix Pho d 2	131	21322677	17
1263	Pistacia vera		Unassigned	Food Plant	Pistacia 11S globulin	472	156001070	19
1264	Pistacia vera		Unassigned	Food Plant	Pistacia 11S globulin	496	110349083	10
1265	Pistacia vera		Unassigned	Food Plant	Pistacia 11S globulin	472	110349085	10
1266	Pistacia vera		Unassigned	Food Plant	Pistacia Pis v 1 2S albumin	149	110349081	10
1267	Pistacia vera		Unassigned	Food Plant	Pistacia Pis v 3 vicilin	519	133711974	10

1268	Pisum sativum	Pea	Pis s 1	Food Plant	Pisum Pis s 1	415	42414629	7
1269	Pisum sativum	Pea	Pis s 1	Food Plant	Pisum Pis s 1	415	42414627	7
1270	Plantago lanceolata	Narrow-leaved plantain	Pla l 1	Aero Plant	Plantago Pla l 1	131	14422359	7
1271	Plantago lanceolata	Narrow-leaved plantain	Pla l 1	Aero Plant	Plantago Pla l 1	131	14422361	7
1272	Plantago lanceolata	Narrow-leaved plantain	Pla l 1	Aero Plant	Plantago Pla l 1	131	14422363	7
1273	Plantago lanceolata	Narrow-leaved plantain	Unassigned	Aero Plant	Plantago Pla l 1	65	29163773	7
1274	Platanus x acerifolia	London plane tree	Unassigned	Aero Plant	Platanus Pla a 1	179	29839547	9
1275	Platanus x acerifolia	London plane tree	Pla a 2	Aero Plant	Platanus Pla a 2	377	49523394	7
1276	Platanus orientalis		Unassigned	Aero Plant	Platanus Pla or 1	170	162949336	9
1277	Plodia interpunctella	Indian meal moth	Unassigned	Aero Insect	Plodia Plo i 1 Arginine kinase	355	15886861	7
1278	Poa pratensis	Kentucky bluegrass	Unassigned	Aero Plant	Poa group II	122	4007655	7
1279	Poa pratensis	Kentucky bluegrass	Poa p 1	Aero Plant	Poa Poa p 1	20	280414	7
1280	Poa pratensis	Kentucky bluegrass	Poa p 1	Aero Plant	Poa Poa p 1	26	320620	7
1281	Poa pratensis	Kentucky bluegrass	Poa p 1	Aero Plant	Poa Poa p 1	263	4090265	7
1282	Poa pratensis	Kentucky bluegrass	Poa p 5	Aero Plant	Poa Poa p 5	303	11991227	7
1283	Poa pratensis	Kentucky bluegrass	Unassigned	Aero Plant	Poa Poa p 9	373	113560	7
1284	Poa pratensis	Kentucky bluegrass	Unassigned	Aero Plant	Poa Poa p 9	307	113562	7
1285	Poa pratensis	Kentucky bluegrass	Unassigned	Aero Plant	Poa Poa p 9	131	539056	7
1286	Poa pratensis	Kentucky bluegrass	Unassigned	Aero Plant	Poa Poa p 9	333	113561	7
1287	Polistes annularis	Paper wasp	Pol a 1	Venom or Salivary	Polistes Pol a 1	301	14423833	7
1288	Polistes dominulus	Paper wasp	Unassigned	Venom or Salivary	Polistes Pol a 1	316	45510893	7
1289	Polistes dominulus	Paper wasp	Unassigned	Venom or Salivary	Polistes Pol a 1	316	45510891	7
1290	Polistes dominulus	Paper wasp	Unassigned	Venom or Salivary	Polistes Pol a 1	316	45510889	7
1291	Polistes	Paper wasp	Unassigned	Venom or	Polistes Pol a 1	337	45510887	7

	dominulus			Salivary				
1292	Polistes gallicus	Paper wasp	Unassigned	Venom or Salivary	Polistes Pol a 1	42	41017429	7
1293	Polistes annularis	Paper wasp	Pol a 2	Venom or Salivary	Polistes Pol a 2	367	14423735	7
1294	Polistes annularis	Paper wasp	Pol a 5	Venom or Salivary	Polistes Venom allergen 5	209	160780	7
1295	Polistes dominulus	Paper wasp	Pol d 5	Venom or Salivary	Polistes Venom allergen 5	227	51093377	7
1296	Polistes exclamans	Paper wasp	Pol e 5	Venom or Salivary	Polistes Venom allergen 5	205	549187	7
1297	Polistes exclamans	Paper wasp	Unassigned	Venom or Salivary	Polistes Venom allergen 5	226	51093375	7
1298	Polistes fuscatus	Paper wasp	Pol f 5	Venom or Salivary	Polistes Venom allergen 5	205	549188	7
1299	Polistes gallicus	Paper wasp	Pol g 5	Venom or Salivary	Polistes Venom allergen 5	206	25091511	7
1300	Polistes dominulus	Paper wasp	Unassigned	Venom or Salivary	Polistes Venom serine protease	277	30909091	7
1301	Polybia paulista	wasp	Unassigned	Venom or Salivary	Polybia p hyaluronidase	345	302201583	12
1302	Polybia paulista	wasp	Unassigned	Venom or Salivary	Polybia p hyaluronidase	288	302425085	12
1303	Polybia paulista	wasp	Unassigned	Venom or Salivary	Polybia p venom allergen 5	141	290792375	11
1304	Polybia paulista	wasp	Unassigned	Venom or Salivary	Polybia p venom allergen 5	207	302595972	12
1305	Polybia paulista	wasp	Pol p 1.0101	Venom or Salivary	Polybia Pol p 1.0101 phospholipase	322	166216292	19
1306	Polybia paulista	wasp	Unassigned	Venom or Salivary	Polybia Pol p 1.0101 phospholipase	302	315190620	12
1307	Protortonia cacti		Unassigned	Food Animal	Protortonia	335	237769615	11
1308	Prunus dulcis x Prunus persica		Unassigned	Food Plant	Prunus Almond-peach hybr profilin Pru 4	131	190613933	10
1309	Prunus dulcis x Prunus persica		Unassigned	Food Plant	Prunus Almond-peach hybr profilin Pru 4	131	190613937	10
1310	Prunus dulcis x Prunus persica		Pru p 2.0201	Food Plant	Prunus persica Pru p 2	246	190613907	10
1311	Prunus dulcis x Prunus persica		Pru p 2.0101	Food Plant	Prunus persica Pru p 2	246	190613911	10
1312	Prunus dulcis x Prunus persica		Pru p 2.0301	Food Plant	Prunus persica Pru p 2	242	190613903	10
1313	Prunus avium	Cherry	Pru av 1	Food Plant	Prunus PRP (Bet v 1 family)	160	1513216	7

1314	Prunus avium	Cherry	Pru av 1	Food Plant	Prunus PRP (Bet v 1 family)	160	44409496	17
1315	Prunus avium	Cherry	Pru av 1	Food Plant	Prunus PRP (Bet v 1 family)	160	44409474	17
1316	Prunus avium	Cherry	Pru av 1	Food Plant	Prunus PRP (Bet v 1 family)	160	44409451	17
1317	Prunus avium	Cherry	Unassigned	Food Plant	Prunus PRP (Bet v 1 family)	159	159162378	19
1318	Prunus persica	Peach	Unassigned	Food Plant	Prunus PRP (Bet v 1 family)	160	82492265	17
1319	Prunus armeniaca	Apricot	Unassigned	Food Plant	Prunus Pru 3	119	313575730	12
1320	Prunus armeniaca	Apricot	Unassigned	Food Plant	Prunus Pru 3	117	313575732	12
1321	Prunus armeniaca	Apricot	Unassigned	Food Plant	Prunus Pru 3	117	313575734	12
1322	Prunus armeniaca	Apricot	Unassigned	Food Plant	Prunus Pru 3	117	313575736	12
1323	Prunus avium	Cherry	Pru av 3	Food Plant	Prunus Pru 3	117	6715520	17
1324	Prunus avium	Cherry	Unassigned	Food Plant	Prunus Pru 3	117	313575726	12
1325	Prunus avium	Cherry	Unassigned	Food Plant	Prunus Pru 3	117	313575728	12
1326	Prunus domestica	Plum	Pru d 3	Food Plant	Prunus Pru 3	91	9297015	17
1327	Prunus persica	Peach	Pru p 3	Food Plant	Prunus Pru 3	91	3287877	17
1328	Prunus persica	Peach	Pru p 3	Food Plant	Prunus Pru 3	92	83754241	17
1329	Prunus persica	Peach	Unassigned	Food Plant	Prunus Pru 3	117	54793477	17
1330	Prunus persica	Peach	Unassigned	Food Plant	Prunus Pru 3	117	313575718	12
1331	Prunus avium	Cherry	Pru av 4	Food Plant	Prunus Pru 4 Profilin	131	4761582	17
1332	Prunus dulcis	Almond	Pru du 4	Food Plant	Prunus Pru 4 Profilin	131	24473794	17
1333	Prunus persica	Peach	Pru p 4.01	Food Plant	Prunus Pru 4 Profilin	131	27528310	17
1334	Prunus persica	Peach	Pru p 4.02	Food Plant	Prunus Pru 4 Profilin	131	27528312	17
1335	Prunus avium	Cherry	Pru av 2	Food Plant	Prunus Pru av 2	245	1144346	17
1336	Prunus dulcis	Almond	Unassigned	Food Plant	Prunus Pru du 6 Amandin Prunus Seed	531	258588247	11
1337	Prunus dulcis	Almond	Unassigned	Food Plant	allergenic protein 2 (Conglutin gamma)	25	75107131	18
1338	Pseudocardium sachalinensis		Unassigned	Food Animal	Pseudocardium tropomyosin	284	219806598	10
1339	Pyrus communis	Pear	Pyr c 1	Food Plant	Pyrus Pyr c 1	159	3044216	17
1340	Pyrus communis	Pear	Pyr c 4	Food Plant	Pyrus Pyr c 4	131	4761580	17
1341	Pyrus communis	Pear	Pyr c 5	Food Plant	Pyrus Pyr c 5	308	3243234	17

1342	Quercus alba	Oak	Que a 1	Aero Plant	Quercus Que a I	24	543675	7
1343	Quercus alba	Oak	Unassigned	Aero Plant	Quercus Que a I	159	167472847	10
1344	Quercus alba	Oak	Unassigned	Aero Plant	Quercus Que a I	160	167472849	10
1345	Rana esculenta	Frog	Ran e 1	Food Animal	Rana Ran e 1	110	20796729	7
1346	Rana sp. CH-2001	Frog	Unassigned	Food Animal	Rana Ran e 1	110	20796733	7
1347	Rana esculenta	Frog	Ran e 2	Food Animal	Rana Ran e 2	109	20797081	7
1348	Rana sp. CH-2001	Frog	Unassigned	Food Animal	Rana Ran e 2	109	20797085	7
1349	Rattus norvegicus	Rat	Rat n 1	Aero Animal	Rattus Rat n 1	181	127533	7
1350	Rattus norvegicus	Rat	Rat n 1	Aero Animal	Rattus Rat n 1	181	81890324	7
1351	Rattus norvegicus	Rat	Unassigned	Aero Animal	Rattus Rat n 1	181	109474987	8
1352	Rhodotorula mucilaginosa	Fungus	Unassigned	Aero Fungi	Rhodotorula Rho m 1	439	37078092	7
1353	Rhodotorula mucilaginosa	Fungus	Unassigned	Aero Fungi	Rhodotorula Rho m 2	342	54654335	7
1354	Ricinus communis	Castor bean	Ric c 1	Food Plant	Ricinus Ric c 1	258	112762	7
1355	Rubus idaeus		Unassigned	Food Plant	Rubus putative allergen Rub i 1	137	110180525	8
1356	Rubus idaeus		Unassigned	Food Plant	Rubus putative allergen Rub i 3	117	110180523	8
1357	Salmo salar	Salmon	Sal s 1	Food Animal	Salmo Sal s 1	109	2493445	7
1358	Salmo salar	Salmon	Sal s 1	Food Animal	Salmo Sal s 1	108	18281421	7
1359	Salmo salar	Salmon	Unassigned	Food Animal	Salmo Sal s 1	109	209734468	10
1360	Salsola kali	Thistle	Unassigned	Aero Plant	Salsola pectin methylesterase Sal k 1.01 & 1.02	362	51242679	8
1361	Salsola kali	Thistle	Unassigned	Aero Plant	Salsola pectin methylesterase Sal k 1.01 & 1.02	339	59895728	8
1362	Salsola kali	Thistle	Unassigned	Aero Plant	Salsola pectin methylesterase Sal k 1.01 & 1.02	339	59895730	8
1363	Salsola kali	Thistle	Unassigned	Aero Plant	Salsola pectin methylesterase Sal k 1.01 & 1.02	339	225810597	10
1364	Salsola kali	Thistle	Sal k 1	Aero Plant	Salsola Sal k 1	11	25090948	7

1365	Salsola kali	Thistle	Sal k 1	Aero Plant	Salsola Sal k 1	8	25090949	7
1366	Salsola kali	Thistle	Sal k 1	Aero Plant	Salsola Sal k 1	9	25090950	7
1367	Salsola kali	Thistle	Sal k 1	Aero Plant	Salsola Sal k 1	14	25090951	7
1368	Salsola kali	Thistle	Unassigned	Aero Plant	Salsola Sal k 3 pollen allergen	757	225810599	10
1369	Salsola kali	Thistle	Unassigned	Aero Plant	Salsola Sal k 4 profilin	133	239916566	11
1370	Salvelinus fontinalis	Brook trout	Unassigned	Food Animal	Salvelinus parvalbumin	109	288557438	11
1371	Salvelinus fontinalis	Brook trout	Unassigned	Food Animal	Salvelinus parvalbumin	108	288557440	11
1372	Sarcoptes scabiei type hominis	Scabies mite	Unassigned	Venom or Salivary	Sarcoptes Apolipoprotein Ssag1.2	330	27462848	7
1373	Sarcoptes scabiei type hominis	Scabies mite	Unassigned	Venom or Salivary	Sarcoptes cysteine protease C08	340	46406002	7
1374	Sarcoptes scabiei type hominis	Scabies mite	Unassigned	Venom or Salivary	Sarcoptes cysteine proteases F04	338	46406012	7
1375	Sarcoptes scabiei type hominis	Scabies mite	Unassigned	Venom or Salivary	Sarcoptes cysteine proteases F04	339	46406014	7
1376	Sarcoptes scabiei type hominis	Scabies mite	Unassigned	Venom or Salivary	Sarcoptes cysteine proteases F04	273	46406016	7
1377	Sarcoptes scabiei type hominis	Scabies mite	Unassigned	Venom or Salivary	Sarcoptes Glutathione S-transferase Mu	219	27462836	7
1378	Sarcoptes scabiei type hominis	Scabies mite	Unassigned	Venom or Salivary	Sarcoptes Glutathione S-transferase Mu	219	60920770	7
1379	Sardinops sagax		Unassigned	Food Animal	Sardinops Sar sa 1 parvalbumin	109	193247972	10
1380	Scapharca broughtonii		Unassigned	Food Animal	Scapharca tropomyosin	284	219806592	10
1381	Schistosoma japonicum	Schistosoma	Unassigned	Protozoan	Schistosoma Putative profilin	129	29841461	7
1382	Schistosoma japonicum	Schistosoma	Unassigned	Protozoan	Schistosoma tegumental antigen	191	2739154	7
1383	Scomber japonicus	Chub mackerel	Unassigned	Food Animal	Scomber Parvalbumin	109	29420793	7
1384	Scomber scombrus	Atlantic mackerel	Unassigned	Food Animal	Scomber Parvalbumin	109	288557436	11
1385	Secale cereale	Rye	Unassigned	Aero Plant	Secale 30K pollen grp 5	16	75140047	7
1386	Secale cereale	Rye	Unassigned	Food Plant	Secale 30K pollen grp 5	292	332205751	12

1387	Secale cereale	Rye	Sec c 1	Aero Plant	Secale sec c 1	26	75198875	17
1388	Secale cereale	Rye	Unassigned	Aero Plant	Secale Sec c 4	520	55859456	17
1389	Secale cereale	Rye	Unassigned	Aero Plant	Secale Sec c 4	518	55859454	17
1390	Sepia esculenta		Unassigned	Food Animal	Sepia tropomyosin	284	83715928	17
1391	Sepioteuthis lessoniana		Unassigned	Food Animal	Sepioteuthis tropomyosin	284	83715930	17
1392	Sesamum indicum	Sesame	Ses i 1	Food Plant	Sesamum Ses i 1	153	13183175	17
1393	Sesamum indicum	Sesame	Unassigned	Food Plant	Sesamum Ses i 1	153	209165427	110
1394	Sesamum indicum	Sesame	Ses i 2	Food Plant	Sesamum Ses i 2	148	5381323	17
1395	Sesamum indicum	Sesame	Ses i 3	Food Plant	Sesamum Ses i 3	585	13183177	17
1396	Sesamum indicum	Sesame	Unassigned	Food Plant	Sesamum Ses i 5	145	198250343	110
1397	Sesamum indicum	Sesame	Unassigned	Food Plant	Sesamum Ses i 5	145	75315271	110
1398	Sinapis alba	White mustard	Sin a 1	Food Plant	Sinapis Sin a 1.01	145	1009434	17
1399	Sinapis alba	White mustard	Sin a 1	Food Plant	Sinapis Sin a 1.01	145	1009436	17
1400	Sinapis alba	White mustard	Sin a 1	Food Plant	Sinapis Sin a 1.01	145	1009438	17
1401	Sinapis alba	White mustard	Sin a 1	Food Plant	Sinapis Sin a 1.01	145	1009440	17
1402	Sinapis alba	White mustard	Sin a 1	Food Plant	Sinapis Sin a 1.01	145	1009442	17
1403	Sinapis alba	White mustard	Sin a 1	Food Plant	Sinapis Sin a 1.01	145	51338758	17
1404	Sinapis alba	White mustard	Sin a 2.0101	Food Plant	Sinapis Sin a 2.01 11S globulin	510	62240390	17
1405	Sinapis alba	White mustard	Unassigned	Food Plant	Sinapis Sin a 2.01 11S globulin	523	62240392	17
1406	Sinapis alba	White mustard	Sin a 3.0101	Food Plant	Sinapis Sin a 3.01 LTP	92	156778059	12
1407	Sinapis alba	White mustard	Sin a 4.0101	Food Plant	Sinapis Sin a 4.01 profilin	131	156778061	12
1408	Solanum tuberosum	Potato	Unassigned	Food Plant	Solanum profilin-like	131	77416979	17
1409	Solanum tuberosum	Potato	Unassigned	Food Plant	Solanum profilin-like	131	77999277	17
1410	Solanum tuberosum	Potato	Unassigned	Food Plant	Solanum Sola t 1	386	21510	17
1411	Solanum tuberosum	Potato	Unassigned	Food Plant	Solanum Sola t 1	386	21512	17
1412	Solanum tuberosum	Potato	Unassigned	Food Plant	Solanum Sola t 1	386	21514	17
1413	Solanum tuberosum	Potato	Unassigned	Food Plant	Solanum Sola t 1	386	169500	17
1414	Solanum tuberosum	Potato	Sola t 1	Food Plant	Solanum Sola t 1	386	158517845	19

1415	Solanum tuberosum	Potato	Sola t 2	Food Plant	Solanum Sola t 2	188	124148	7
1416	Solanum tuberosum	Potato	Sola t 3	Food Plant	Solanum Sola t 3	222	20141344	7
1417	Solanum tuberosum	Potato	Unassigned	Food Plant	Solanum Sola t 4	217	21413	7
1418	Solanum tuberosum	Potato	Sola t 4	Food Plant	Solanum Sola t 4	221	20141714	7
1419	Solen strictus		Unassigned	Food Animal	Solen tropomyosin	284	219806602	10
1420	Solenopsis invicta	Red fire ant	Unassigned	Venom or Salivary	Solenopsis Sol i 1	58	1336809	7
1421	Solenopsis invicta	Red fire ant	Unassigned	Venom or Salivary	Solenopsis Sol i 1	25	1336811	7
1422	Solenopsis invicta	Red fire ant	Unassigned	Venom or Salivary	Solenopsis Sol i 1	26	1336812	7
1423	Solenopsis invicta	Red fire ant	Unassigned	Venom or Salivary	Solenopsis Sol i 1	26	1336813	7
1424	Solenopsis invicta	Red fire ant	Unassigned	Venom or Salivary	Solenopsis Sol i 1	346	51093373	7
1425	Solenopsis invicta	Red fire ant	Sol i 2	Venom or Salivary	Solenopsis Sol i and Sol r Venom allergen II	138	549179	7
1426	Solenopsis richteri	Black fire ant	Unassigned	Venom or Salivary	Solenopsis Sol i and Sol r Venom allergen II	119	6136162	7
1427	Solenopsis invicta	Red fire ant	Sol i 3	Venom or Salivary	Solenopsis Venom allergen III	234	14424466	7
1428	Solenopsis richteri	Black fire ant	Unassigned	Venom or Salivary	Solenopsis Venom allergen III	211	6136163	7
1429	Solenopsis geminata	Tropical Fire Ant	Sol g 4	Venom or Salivary	Solenopsis Venom allergen IV	137	7638028	7
1430	Solenopsis geminata	Tropical Fire Ant	Sol g 4	Venom or Salivary	Solenopsis Venom allergen IV	137	7638030	7
1431	Solenopsis invicta	Red fire ant	Sol i 4	Venom or Salivary	Solenopsis Venom allergen IV	137	4038411	7
1432	Solenopsis invicta	Red fire ant	Sol i 4	Venom or Salivary	Solenopsis Venom allergen IV	137	14424465	7
1433	Solenopsis saevisima	Brazilian fire ant	Unassigned	Venom or Salivary	Solenopsis Venom allergen IV	137	291092710	12
1434	Staphylococcus laureus		Unassigned	Bacteria skin	Staphylococcus enterotoxin SEA	233	1633233	9
1435	Staphylococcus laureus		Unassigned	Bacteria skin	Staphylococcus enterotoxin SEB	254	83308249	9
1436	Staphylococcus laureus		Unassigned	Bacteria skin	Staphylococcus enterotoxin SEC	266	462026	9

1437	Staphylococcus aureus		Unassigned	Bacteria skin	Staphylococcus enterotoxin SED	258	119654	9
1438	Staphylococcus aureus		Unassigned	Bacteria skin	Staphylococcus enterotoxin TSST 1	234	136457	9
1439	Suidasia medanensis		Unassigned	Aero Mite	Suidasia putative Sui m 2	141	45738062	7
1440	Sus scrofa	Pig	Unassigned	Aero Animal	Sus Porcine Pepsin	385	118572685	11
1441	Syringa vulgaris	Lilac	Syr v 1.0101	Aero Plant	Syringa Syr v I	145	631911	7
1442	Syringa vulgaris	Lilac	Syr v 1.0102	Aero Plant	Syringa Syr v I	145	631912	7
1443	Syringa vulgaris	Lilac	Syr v 1.0103	Aero Plant	Syringa Syr v I	145	631913	7
1444	Tabanus yao	Horse Fly	Tab y 1.0101	Venom or Salivary	Tabanus Tab y 1 Apyrase	554	323473390	12
1445	Tabanus yao	Horse Fly	Tab y 2.0101	Venom or Salivary	Tabanus Tab y 2 Hyaluronidase	349	304273371	12
1446	Tabanus yao	Horse Fly	Tab y 5.0101	Venom or Salivary	Tabanus Tab y 5	256	304273369	12
1447	Thaumetopoea pityocampa	Pine moth	Unassigned	Contact	Thaumetopoea Tha p 1	126	301030229	12
1448	Theragra chalcogramma	Alaska pollock	Unassigned	Food Animal	Theragra parvalbumin	109	14531020	7
1449	Theragra chalcogramma	Alaska pollock	Unassigned	Food Animal	Theragra parvalbumin	109	14531018	7
1450	Todarodes pacificus	Japanese flying squid	Unassigned	Food Animal	Todarodes tropomyosin	284	83715932	7
1451	Trachurus japonicus		Unassigned	Food Animal	Trachurus parvalbumin	107	77799800	7
1452	Tresus keenae		Unassigned	Food Animal	Tresus tropomyosin	284	219806600	10
1453	Triatoma protracta	Western conenose	Tria p 1	Venom or Salivary	Triatoma Tria p 1	169	15426413	7
1454	Arthroderma benhamiae	Fungus	Unassigned	Contact	Trichophyton (Arthroderma) Tri m 2	292	23894240	7
1455	Arthroderma benhamiae	Fungus	Unassigned	Contact	Trichophyton (Arthroderma) Tri m 2	404	23894244	7
1456	Trichophyton rubrum	Fungus	Tri r 2	Contact	Trichophyton (Arthroderma) Tri m 2	412	5813790	7
1457	Trichophyton schoenleinii	Fungus	Unassigned	Contact	Trichophyton (Arthroderma) Tri m 2	405	74663809	12
1458	Arthroderma benhamiae	Fungus	Unassigned	Contact	Trichophyton (Arthroderma) Tri m 2	726	23894232	7

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1459	Arthroderma vanbreuseghemii	Fungus	Unassigned	Contact	Trichophyton (Arthroderma) Tri m 4	726	219687753	10
1460	Trichophyton rubrum	Fungus	Tri r 4	Contact	Trichophyton tri 4 allergen (Arthroderma)	726	5813788	7
1461	Trichophyton schoenleinii	Fungus	Unassigned	Contact	Trichophyton tri 4 allergen (Arthroderma)	726	23894227	7
1462	Triticum laestivum	Wheat	Unassigned	Aero Plant	Triticum Tri a 14 LTP_amylase inhibitor	113	417370	11
1463	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum 5a2 protein	94	66840998	7
1464	Triticum laestivum	Wheat	Unassigned	Aero Plant	Triticum aAI CM16_17	143	195957140	10
1465	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum aAI CM16_17	143	21711	7
1466	Triticum turgidum	Wheat	Unassigned	Aero Plant	Triticum aAI CM16_17	18	244610	7
1467	Triticum turgidum subsp. durum	Wheat	Unassigned	Food Plant	Triticum aAI CM16_17	143	21916	7
1468	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum aAI CM3	168	21713	7
1469	Triticum turgidum subsp. durum	Wheat	Unassigned	Food Plant	Triticum aAI CM3	168	100834	7
1470	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum Alpha/beta gliadin IgE & celiac	286	21755	7
1471	Triticum laestivum	Wheat	Unassigned	Gliadin	Triticum Alpha/beta gliadin IgE & celiac	307	21673	7
1472	Triticum laestivum	Wheat	Unassigned	Gliadin	Triticum Alpha/beta gliadin IgE & celiac	296	21757	7
1473	Triticum laestivum	Wheat	Unassigned	Gliadin	Triticum Alpha/beta gliadin IgE & celiac	286	21761	7
1474	Triticum laestivum	Wheat	Unassigned	Gliadin	Triticum Alpha/beta gliadin IgE & celiac	313	21765	7
1475	Triticum laestivum	Wheat	Unassigned	Gliadin	Triticum Alpha/beta gliadin IgE & celiac	318	170710	7
1476	Triticum laestivum	Wheat	Unassigned	Gliadin	Triticum Alpha/beta gliadin IgE & celiac	291	170712	7

1477	Triticum aestivum	Wheat	Unassigned	Gliadin	Triticum Alpha/beta gliadin IgE & celiac	313	170718	7
1478	Triticum aestivum	Wheat	Unassigned	Gliadin	Triticum Alpha/beta gliadin IgE & celiac	286	170720	7
1479	Triticum aestivum	Wheat	Unassigned	Gliadin	Triticum Alpha/beta gliadin IgE & celiac	262	170722	7
1480	Triticum aestivum	Wheat	Unassigned	Gliadin	Triticum Alpha/beta gliadin IgE & celiac	297	170724	7
1481	Triticum aestivum	Wheat	Unassigned	Gliadin	Triticum Alpha/beta gliadin IgE & celiac	282	170726	7
1482	Triticum aestivum	Wheat	Unassigned	Gliadin	Triticum Alpha/beta gliadin IgE & celiac	186	170728	7
1483	Triticum aestivum	Wheat	Unassigned	Gliadin	Triticum Alpha/beta gliadin IgE & celiac	287	473876	7
1484	Triticum aestivum	Wheat	Unassigned	Gliadin	Triticum Alpha/beta gliadin IgE & celiac	259	1304264	7
1485	Triticum urartu	Wheat	Unassigned	Food Plant	Triticum Alpha/beta gliadin IgE & celiac	296	170740	7
1486	Triticum aestivum	Wheat	Unassigned	Aero Plant	Triticum Bakers asthma allergen #4	27	3913017	7
1487	Triticum aestivum	Wheat	Unassigned	Gliadin	Triticum gamma gliadin IgE & celiac	302	170702	7
1488	Triticum aestivum	Wheat	Unassigned	Gliadin	Triticum gamma gliadin IgE & celiac	291	170708	7
1489	Triticum aestivum	Wheat	Unassigned	Gliadin	Triticum gamma gliadin IgE & celiac	304	170730	7
1490	Triticum aestivum	Wheat	Unassigned	Gliadin	Triticum gamma gliadin IgE & celiac	323	170732	7
1491	Triticum aestivum	Wheat	Unassigned	Gliadin	Triticum gamma gliadin IgE & celiac	244	170734	7
1492	Triticum aestivum	Wheat	Unassigned	Gliadin	Triticum gamma gliadin IgE & celiac	251	170736	7
1493	Triticum aestivum	Wheat	Unassigned	Gliadin	Triticum gamma gliadin IgE & celiac	327	170738	7
1494	Triticum aestivum	Wheat	Unassigned	Gliadin	Triticum gamma gliadin IgE & celiac	279	1063270	7

					celiac			
1495	Triticum laestivum	Wheat	Unassigned	Gliadin	Triticum gamma gliadin IgE & celiac	285	62484809	7
1496	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum HMW glutenin	830	21743	7
1497	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum HMW glutenin	648	21751	7
1498	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum HMW glutenin	660	21779	7
1499	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum HMW glutenin	39	21793	7
1500	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum HMW glutenin	705	22090	7
1501	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum HMW glutenin	815	170743	7
1502	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum HMW glutenin	838	736319	7
1503	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum HMW glutenin	101	897811	7
1504	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum LMW glutenin	307	21773	7
1505	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum LMW glutenin	356	21783	7
1506	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum LMW glutenin	373	75317968	7
1507	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum LMW glutenin	229	886963	7
1508	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum LMW glutenin	261	886965	7
1509	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum LMW glutenin	276	886967	7
1510	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum LMW glutenin	285	75219081	7
1511	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum LMW glutenin	326	62550933	7
1512	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum LMW glutenin	369	335331566	12
1513	Triticum turgidum subsp. durum	Wheat	Unassigned	Food Plant	Triticum LMW glutenin	295	21926	7
1514	Triticum turgidum subsp. durum	Wheat	Unassigned	Food Plant	Triticum LMW glutenin	285	21930	7
1515	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum omega-5 gliadin Tri a 19	439	73912496	7
1516	Triticum	Wheat	Unassigned	Food Plant	Triticum omega-5	359	208605344	10

	laestivum				gliadin Tri a 19			
1517	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum omega-5 gliadin Tri a 19	272	208605346	10
1518	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum omega-5 gliadin Tri a 19	346	208605348	10
1519	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum Profilin	141	1008443	7
1520	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum Profilin	140	1008445	7
1521	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum Profilin	138	1052817	7
1522	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum Profilin	131	190684061	11
1523	Triticum laestivum	Wheat	Unassigned	Aero Plant	Triticum putative flour allergens Constantin 2010	118	190684055	11
1524	Triticum laestivum	Wheat	Unassigned	Aero Plant	Triticum putative flour allergens Constantin 2010	222	190684057	11
1525	Triticum laestivum	Wheat	Unassigned	Aero Plant	Triticum putative flour allergens Constantin 2010	218	190684059	11
1526	Triticum laestivum	Wheat	Unassigned	Aero Plant	Triticum putative flour allergens Constantin 2010	213	190684063	11
1527	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum putative leucine-rich repeat protein	137	66840996	7
1528	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum serine carboxypeptidase II	260	66840994	7
1529	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum serine carboxypeptidase II	444	125987805	10
1530	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum Serine protease inhibitor	399	1885350	7
1531	Triticum laestivum	Wheat	Unassigned	Aero Plant	Triticum serine proteinase inhibitor-like	84	154101366	10
1532	Triticum laestivum	Wheat	Unassigned	Aero Plant	Triticum serine proteinase inhibitor-like	84	122065237	11
1533	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum Thaumatin-like	173	135917	12
1534	Triticum laestivum	Wheat	Unassigned	Aero Plant	Triticum Tri a 29	120	253783731	11
1535	Triticum laestivum	Wheat	Unassigned	Aero Plant	Triticum Tri a 29	120	283465827	11
1536	Triticum laestivum	Wheat	Unassigned	Food Plant	Triticum Tri a 29	145	21701	7

1537	Triticum turgidum subsp. durum	Wheat	Unassigned	Food Plant	Triticum Tri a 29	145	21920	7
1538	Triticum aestivum	Wheat	Unassigned	Food Plant	Triticum Triosephosphate isomerase	253	11124572	7
1539	Tyrophagus putrescentiae	Dust mite	Unassigned	Aero Mite	Tyrophagus Tyr p 10 tropomyosin	284	148615631	9
1540	Tyrophagus putrescentiae	Dust mite	Unassigned	Aero Mite	Tyrophagus Tyr p 10 tropomyosin	201	156938915	9
1541	Tyrophagus putrescentiae	Dust mite	Unassigned	Aero Mite	Tyrophagus Tyr p 10 tropomyosin	284	48249227	9
1542	Tyrophagus putrescentiae	Dust mite	Tyr p 13	Aero Mite	Tyrophagus Tyr p 13	131	51860756	7
1543	Tyrophagus putrescentiae	Dust mite	Unassigned	Aero Mite	Tyrophagus Tyr p 13	130	121296500	9
1544	Tyrophagus putrescentiae	Dust mite	Unassigned	Aero Mite	Tyrophagus Tyr p 13	131	156938917	9
1545	Tyrophagus putrescentiae	Dust mite	Unassigned	Aero Mite	Tyrophagus Tyr p 2	141	3182907	9
1546	Tyrophagus putrescentiae	Dust mite	Unassigned	Aero Mite	Tyrophagus Tyr p 24 Troponin C	153	219815476	11
1547	Tyrophagus putrescentiae	Dust mite	Unassigned	Aero Mite	Tyrophagus Tyr p 3	285	167540622	11
1548	Vespa crabro	European hornet	Vesp c 5	Venom or Salivary	Vespa Venom allergen 5 hornets	202	549184	7
1549	Vespa crabro	European hornet	Vesp c 5	Venom or Salivary	Vespa Venom allergen 5 hornets	202	549185	7
1550	Vespa mandarinia	Wasp	Vesp m 5	Venom or Salivary	Vespa Venom allergen 5 hornets	202	6136165	7
1551	Vespa crabro	European hornet	Unassigned	Venom or Salivary	Vespa Vesp c 1 phospholipase	301	313471397	12
1552	Vespula germanica	Wasp	Unassigned	Venom or Salivary	Vespula Phospholipase A1- Ves m/v 1	300	74035843	7
1553	Vespula maculifrons	Wasp	Ves m 1	Venom or Salivary	Vespula Phospholipase A1- Ves m/v 1	300	1709545	8
1554	Vespula vulgaris	Wasp	Ves v 1	Venom or Salivary	Vespula Phospholipase A1- Ves m/v 1	336	897647	7
1555	Vespula flavopilosa	Wasp	Ves f 5	Venom or Salivary	Vespula Venom allergen 5 yellow jackets	204	549189	7
1556	Vespula germanica	Wasp	Ves g 5	Venom or Salivary	Vespula Venom allergen 5 yellow jackets	204	549190	7
1557	Vespula germanica	Wasp	Unassigned	Venom or Salivary	Vespula Venom allergen 5 yellow	204	74035841	7

					jackets			
1558	Vespula maculifrons	Wasp	Ves m 5	Venom or Salivary	Vespula Venom allergen 5 yellow jackets	204	549191	7
1559	Vespula maculifrons	Wasp	Unassigned	Venom or Salivary	Vespula Venom allergen 5 yellow jackets	227	85681830	7
1560	Vespula pensylvanica	Wasp	Ves p 5	Venom or Salivary	Vespula Venom allergen 5 yellow jackets	204	549192	7
1561	Vespula squamosa	Wasp	Ves s 5	Venom or Salivary	Vespula Venom allergen 5 yellow jackets	205	549193	7
1562	Vespula vidua	Wasp	Ves vi 5	Venom or Salivary	Vespula Venom allergen 5 yellow jackets	206	549194	7
1563	Vespula vulgaris	Wasp	Ves v 5	Venom or Salivary	Vespula Venom allergen 5 yellow jackets	227	162551	7
1564	Vespula vulgaris	Wasp	Ves v 5	Venom or Salivary	Vespula Venom allergen 5 yellow jackets	204	4826574	7
1565	Vespula vulgaris	Wasp	Ves v 5	Venom or Salivary	Vespula Venom allergen 5 yellow jackets	209	11514279	7
1566	Vespula maculifrons	Wasp	Unassigned	Venom or Salivary	Vespula Ves m 2 Hyaluronidase	31	313118253	12
1567	Vespula squamosa	Wasp	Unassigned	Venom or Salivary	Vespula Ves s 1 phospholipase	298	313471398	12
1568	Vespula germanica	Wasp	Unassigned	Venom or Salivary	Vespula Ves v 2	331	116174180	8
1569	Vespula germanica	Wasp	Unassigned	Venom or Salivary	Vespula Ves v 2	323	116174182	8
1570	Vespula vulgaris	Wasp	Ves v 2	Venom or Salivary	Vespula Ves v 2	331	1346323	7
1571	Vespula vulgaris	Wasp	Unassigned	Venom or Salivary	Vespula Ves v 2	340	62147665	7
1572	Vespula vulgaris	Wasp	Unassigned	Venom or Salivary	Vespula Ves v 2	331	109157163	8
1573	Vespula vulgaris	Wasp	Unassigned	Venom or Salivary	Vespula Ves v 3 dipeptidylpeptidase IV	776	313471718	12
1574	Vigna radiata		Unassigned	Food Plant	Vigna Vig r 1 PR 10	155	60418924	7
1575	Vitis sp.	Grape	Unassigned	Food Plant	Vitis Lipid transfer protein P3	91	145559502	8
1576	Vitis sp.	Grape	Vit v 1	Food Plant	Vitis Vit v 1 LTP	37	462719	7
1577	Vitis sp.	Grape	Unassigned	Food Plant	Vitis Vit v 1 LTP	38	462717	7
1578	Xiphias gladius		Unassigned	Food	Xiphias Xip g 1	109	222352960	10

				Animal	beta-parvalbumin			
1579	Zea mays	Corn	Unassigned	Aero Plant	Zea m 1 isoform	263	89892721	7
1580	Zea mays	Corn	Unassigned	Aero Plant	Zea m 1 isoform	252	89892723	7
1581	Zea mays	Corn	Unassigned	Aero Plant	Zea m 1 isoform	99	105969543	8
1582	Zea mays	Corn	Unassigned	Aero Plant	Zea m 1 isoform	269	105969545	8
1583	Zea mays	Corn	Unassigned	Aero Plant	Zea m 1 isoform	270	115502167	9
1584	Zea mays	Corn	Unassigned	Aero Plant	Zea m 1 isoform	269	115502168	9
1585	Zea mays	Corn	Unassigned	Food Plant	Zea profilin	131	2642324	7
1586	Zea mays	Corn	Unassigned	Food Plant	Zea profilin	131	110644952	8
1587	Zea mays	Corn	Unassigned	Food Plant	Zea profilin	131	110644954	8
1588	Zea mays	Corn	Unassigned	Food Plant	Zea profilin	131	110644956	8
1589	Zea mays	Corn	Unassigned	Food Plant	Zea profilin	131	110644958	8
1590	Zea mays	Corn	Unassigned	Food Plant	Zea profilin	131	110644960	8
1591	Zea mays	Corn	Unassigned	Food Plant	Zea profilin	131	110644962	8
1592	Zea mays	Corn	Unassigned	Food Plant	Zea profilin	130	110644964	8
1593	Zea mays	Corn	Unassigned	Aero Plant	Zea putative Zea m 13?	410	89892725	7
1594	Zea mays	Corn	Unassigned	Aero Plant	Zea putative Zea m 13?	404	89892727	7
1595	Zea mays	Corn	Unassigned	Aero Plant	Zea putative Zea m 13?	411	89892729	7
1596	Zea mays	Corn	Unassigned	Aero Plant	Zea Zea m 13	170	1588669	7
1597	Zea mays	Corn	Zea m 14	Food Plant	Zea Zea m 14	120	128388	7
1598	Zea mays	Corn	Unassigned	Aero Plant	Zea Zea m 25 thioredoxin	128	66841002	7
1599	Zea mays	Corn	Unassigned	Aero Plant	Zea Zea m1	269	28630919	7
1600	Zea mays	Corn	Unassigned	Aero Plant	Zea Zea m1	269	28630923	7
1601	Zea mays	Corn	Unassigned	Aero Plant	Zea Zea m1	269	14193761	8
1602	Zea mays	Corn	Unassigned	Aero Plant	Zea Zea m1	245	114794319	8
1603	Ziziphus mauritiana	Chinese-date	Unassigned	Food Plant	Ziziphus Ziz m 1	330	61225281	7

## D.1 Omitted allergens from allergenonline

A few of the entries were omitted, due to wrong accession codes, unpublished sequences or other errors:

Blomia tropicalis Mite UnassignedAero MiteBlomia Blo t 1.02  
Brassica oleracea Cabbage UnassignedFood PlantBrassica Bra o 3 LTP manual entry

## E List of allergens from allergen.org

List of allergens that have been tested by the EFSA scientific opinion recommended allergen analysis described in section 2. The sequences were downloaded via <http://www.allergen.org>.

Aca s 13.0101 076821 Acarus siro (Storage mite)  
Act c 10.0101 P85204 Actinidia chinensis (Gold Kiwi fruit)  
Act c 5.0101 P85261 Actinidia chinensis (Gold Kiwi fruit)  
Act c 8.0101 D1YSM4 Actinidia chinensis (Gold Kiwi fruit)  
Act d 1.0101 P00785 Actinidia deliciosa (Kiwi fruit)  
Act d 10.0101 P85205 Actinidia deliciosa (Kiwi fruit)  
Act d 10.0201 P85206 Actinidia deliciosa (Kiwi fruit)  
Act d 11.0101 P85524 Actinidia deliciosa (Kiwi fruit)  
Act d 2.0101 P81370 Actinidia deliciosa (Kiwi fruit)  
Act d 3.0101 P85063 Actinidia deliciosa (Kiwi fruit)  
Act d 4.0101 Q6TPK4 Actinidia deliciosa (Kiwi fruit)  
Act d 5.0101 P84527 Actinidia deliciosa (Kiwi fruit)  
Act d 6.0101 P83326 Actinidia deliciosa (Kiwi fruit)  
Act d 7.0101 P85076 Actinidia deliciosa (Kiwi fruit)  
Act d 8.0101 D1YSM5 Actinidia deliciosa (Kiwi fruit)  
Aed a 1.0101 P50635 Aedes aegypti (Yellow fever mosquito)  
Aed a 2.0101 P18153 Aedes aegypti (Yellow fever mosquito)  
Aed a 3.0101 001949 Aedes aegypti (Yellow fever mosquito)  
Aln g 1.0101 P38948 Alnus glutinosa (Alder)  
Aln g 4.0101 081701 Alnus glutinosa (Alder)  
Alt a 1.0101 P79085 Alternaria alternata (Alternaria rot fungus)  
Alt a 1.0102 Q6Q128 Alternaria alternata (Alternaria rot fungus)  
Alt a 10.0101 P42041 Alternaria alternata (Alternaria rot fungus)  
Alt a 12.0101 P49148 Alternaria alternata (Alternaria rot fungus)  
Alt a 13.0101 Q6R4B4 Alternaria alternata (Alternaria rot fungus)  
Alt a 3.0101 P78983 Alternaria alternata (Alternaria rot fungus)  
Alt a 4.0101 Q00002 Alternaria alternata (Alternaria rot fungus)  
Alt a 5.0101 P42037 Alternaria alternata (Alternaria rot fungus)  
Alt a 6.0101 Q9HDT3 Alternaria alternata (Alternaria rot fungus)  
Alt a 7.0101 P42058 Alternaria alternata (Alternaria rot fungus)  
Alt a 8.0101 P0C0Y4 Alternaria alternata (Alternaria rot fungus)  
Ama r 2.0101 C3W2Q7 Amaranthus retroflexus (Redroot pigweed)  
Amb a 1.0101 P27759 Ambrosia artemisiifolia (Short ragweed)  
Amb a 1.0201 P27760 Ambrosia artemisiifolia (Short ragweed)  
Amb a 1.0202 E1XUL3 Ambrosia artemisiifolia (Short ragweed)  
Amb a 1.0301 P27761 Ambrosia artemisiifolia (Short ragweed)  
Amb a 1.0302 P27761 Ambrosia artemisiifolia (Short ragweed)  
Amb a 1.0303 P27761 Ambrosia artemisiifolia (Short ragweed)  
Amb a 1.0304 E1XUL4 Ambrosia artemisiifolia (Short ragweed)  
Amb a 1.0305 E1XUL5 Ambrosia artemisiifolia (Short ragweed)  
Amb a 1.0401 P28744 Ambrosia artemisiifolia (Short ragweed)  
Amb a 1.0402 E1XUL9 Ambrosia artemisiifolia (Short ragweed)  
Amb a 1.0501 P27762 Ambrosia artemisiifolia (Short ragweed)  
Amb a 1.0502 E1XUM1 Ambrosia artemisiifolia (Short ragweed)  
Amb a 10.0101 Q2KN25 Ambrosia artemisiifolia (Short ragweed)  
Amb a 3.0101 P00304 Ambrosia artemisiifolia (Short ragweed)  
Amb a 5.0101 P02878 Ambrosia artemisiifolia (Short ragweed)  
Amb a 6.0101 004004 Ambrosia artemisiifolia (Short ragweed)  
Amb a 8.0101 Q2KN24 Ambrosia artemisiifolia (Short ragweed)  
Amb a 8.0102 Q2KN23 Ambrosia artemisiifolia (Short ragweed)  
Amb a 9.0101 Q2KN27 Ambrosia artemisiifolia (Short ragweed)  
Amb a 9.0102 Q2KN26 Ambrosia artemisiifolia (Short ragweed)  
Amb p 5.0101 P43174 Ambrosia psilostachya (Western ragweed)  
Amb p 5.0201 P43175 Ambrosia psilostachya (Western ragweed)  
Amb t 5.0101 P10414 Ambrosia trifida (Giant ragweed)  
Ana c 1.0101 Q94JN2 Ananas comosus (Pineapple)  
Ana c 2.0101 Q23791 Ananas comosus (Pineapple)  
Ana o 1.0101 Q8L5L5 Anacardium occidentale (Cashew)  
Ana o 1.0102 Q8L5L6 Anacardium occidentale (Cashew)

Ana o 2.0101 Q8GZP6 Anacardium occidentale (Cashew)  
 Ana o 3.0101 Q8H2B8 Anacardium occidentale (Cashew)  
 Ani s 1.0101 Q7Z1K3 Anisakis simplex (Nematode)  
 Ani s 2.0101 Q9NJA9 Anisakis simplex (Nematode)  
 Ani s 3.0101 Q9NAS5 Anisakis simplex (Nematode)  
 Ani s 4.0101 Q14QT4 Anisakis simplex (Nematode)  
 Ani s 5.0101 A1IKL2 Anisakis simplex (Nematode)  
 Ani s 6.0101 A1IKL3 Anisakis simplex (Nematode)  
 Ani s 7.0101 A9XBJ8 Anisakis simplex (Nematode)  
 Ani s 9.0101 B2XCP1 Anisakis simplex (Nematode)  
 Ant o 1.0101 Q7M1X6 Anthoxanthum odoratum (Sweet vernal grass)  
 Api c 1.0101 Q9EMK4 Apis cerana (Eastern hive bee)  
 Api d 1.0101 Q7M4I5 Apis dorsata (Giant honeybee)  
 Api g 1.0101 P49372 Apium graveolens (Celery)  
 Api g 1.0201 P92918 Apium graveolens (Celery)  
 Api g 3.0101 P92919 Apium graveolens (Celery)  
 Api g 4.0101 Q9XF37 Apium graveolens (Celery)  
 Api g 5.0101 P81943 Apium graveolens (Celery)  
 Api m 1.0101 P00630 Apis mellifera (Honey bee)  
 Api m 10.0101 Q1HNN7 Apis mellifera (Honey bee)  
 Api m 2.0101 Q08169 Apis mellifera (Honey bee)  
 Api m 3.0101 Q4TUB9 Apis mellifera (Honey bee)  
 Api m 4.0101 P01501 Apis mellifera (Honey bee)  
 Api m 5.0101 B2D0J4 Apis mellifera (Honey bee)  
 Api m 7.0101 Q8MQS8 Apis mellifera (Honey bee)  
 Api m 8.0101 B2D0J5 Apis mellifera (Honey bee)  
 Api m 9.0101 C9WMM5 Apis mellifera (Honey bee)  
 Ara h 1.0101 P43238 Arachis hypogaea (Peanut)  
 Ara h 10.0101 Q647G5 Arachis hypogaea (Peanut)  
 Ara h 10.0102 Q647G4 Arachis hypogaea (Peanut)  
 Ara h 11.0101 Q45W87 Arachis hypogaea (Peanut)  
 Ara h 2.0101 Q6PSU2 Arachis hypogaea (Peanut)  
 Ara h 2.0201 Q6PSU2 Arachis hypogaea (Peanut)  
 Ara h 3.0101 O82580 Arachis hypogaea (Peanut)  
 Ara h 3.0201 Q9SQH7 Arachis hypogaea (Peanut)  
 Ara h 5.0101 Q9SQI9 Arachis hypogaea (Peanut)  
 Ara h 6.0101 Q647G9 Arachis hypogaea (Peanut)  
 Ara h 7.0101 Q9SQH1 Arachis hypogaea (Peanut)  
 Ara h 7.0201 B4XID4 Arachis hypogaea (Peanut)  
 Ara h 8.0101 Q6VT83 Arachis hypogaea (Peanut)  
 Ara h 8.0201 BOYIU5 Arachis hypogaea (Peanut)  
 Ara h 9.0101 B6CEX8 Arachis hypogaea (Peanut)  
 Ara h 9.0201 B6CG41 Arachis hypogaea (Peanut)  
 Arc s 8.0101 Q8T5G9 Archaeopotamobius sibiricus (Crustacean species)  
 Arg r 1.0101 Q5GQ85 Argas reflexus (Pigeon tick)  
 Art fr 5.0101 A7L499 Artemia franciscana (Brine shrimp)  
 Art v 1.0101 Q84ZX5 Artemisia vulgaris (Mugwort)  
 Art v 2.0101 Q7M1G9 Artemisia vulgaris (Mugwort)  
 Art v 3.0101 P0C088 Artemisia vulgaris (Mugwort)  
 Art v 3.0201 C4MGG9 Artemisia vulgaris (Mugwort)  
 Art v 3.0202 C4MGH0 Artemisia vulgaris (Mugwort)  
 Art v 3.0301 C4MGH1 Artemisia vulgaris (Mugwort)  
 Art v 4.0101 Q8H2C9 Artemisia vulgaris (Mugwort)  
 Art v 4.0201 Q8H2C8 Artemisia vulgaris (Mugwort)  
 Art v 5.0101 A0PJ17 Artemisia vulgaris (Mugwort)  
 Art v 6.0101 A0PJ16 Artemisia vulgaris (Mugwort)  
 Asc l 3.0101 COL3K2 Ascaris lumbricoides (Common roundworm)  
 Asc s 1.0101 Q06811 Ascaris suum (Pig roundworm)  
 Asp f 1.0101 P67875 Aspergillus fumigatus (fungus)  
 Asp f 10.0101 Q12547 Aspergillus fumigatus (fungus)  
 Asp f 11.0101 Q9Y7F6 Aspergillus fumigatus (fungus)  
 Asp f 12.0101 P40292 Aspergillus fumigatus (fungus)  
 Asp f 13.0101 P28296 Aspergillus fumigatus (fungus)  
 Asp f 15.0101 O60022 Aspergillus fumigatus (fungus)  
 Asp f 16.0101 O74682 Aspergillus fumigatus (fungus)  
 Asp f 17.0101 O60025 Aspergillus fumigatus (fungus)  
 Asp f 18.0101 P87184 Aspergillus fumigatus (fungus)  
 Asp f 2.0101 P79017 Aspergillus fumigatus (fungus)  
 Asp f 22.0101 Q96X30 Aspergillus fumigatus (fungus)

Asp f 23.0101 Q8NKF4 *Aspergillus fumigatus* (fungus)  
 Asp f 27.0101 Q4WXX5 *Aspergillus fumigatus* (fungus)  
 Asp f 28.0101 Q1RQJ1 *Aspergillus fumigatus* (fungus)  
 Asp f 29.0101 Q4WV97 *Aspergillus fumigatus* (fungus)  
 Asp f 3.0101 Q43099 *Aspergillus fumigatus* (fungus)  
 Asp f 34.0101 A4FSH5 *Aspergillus fumigatus* (fungus)  
 Asp f 4.0101 Q60024 *Aspergillus fumigatus* (fungus)  
 Asp f 5.0101 P46075 *Aspergillus fumigatus* (fungus)  
 Asp f 6.0101 Q92450 *Aspergillus fumigatus* (fungus)  
 Asp f 7.0101 Q42799 *Aspergillus fumigatus* (fungus)  
 Asp f 8.0101 Q9U0Z6 *Aspergillus fumigatus* (fungus)  
 Asp f 9.0101 Q42800 *Aspergillus fumigatus* (fungus)  
 Asp n 14.0101 Q93933 *Aspergillus niger*  
 Asp n 25.0101 P34754 *Aspergillus niger*  
 Asp o 13.0101 P12547 *Aspergillus oryzae*  
 Asp o 21.0101 P10529 *Aspergillus oryzae*  
 Ber e 1.0101 P04403 *Bertholletia excelsa* (Brazil nut)  
 Ber e 2.0101 Q84ND2 *Bertholletia excelsa* (Brazil nut)  
 Bet v 1.0101 P15494 *Betula verrucosa* (*Betula pendula*) (European white birch)  
 Bet v 1.0102 P43177 *Betula verrucosa* (*Betula pendula*) (European white birch)  
 Bet v 1.0103 P43178 *Betula verrucosa* (*Betula pendula*) (European white birch)  
 Bet v 1.0104 P43179 *Betula verrucosa* (*Betula pendula*) (European white birch)  
 Bet v 1.0105 P43180 *Betula verrucosa* (*Betula pendula*) (European white birch)  
 Bet v 1.0106 P43183 *Betula verrucosa* (*Betula pendula*) (European white birch)  
 Bet v 1.0107 P43185 *Betula verrucosa* (*Betula pendula*) (European white birch)  
 Bet v 1.0108 Q96365 *Betula verrucosa* (*Betula pendula*) (European white birch)  
 Bet v 1.0109 Q96366 *Betula verrucosa* (*Betula pendula*) (European white birch)  
 Bet v 1.0110 Q96367 *Betula verrucosa* (*Betula pendula*) (European white birch)  
 Bet v 1.0111 Q96368 *Betula verrucosa* (*Betula pendula*) (European white birch)  
 Bet v 1.0112 P15494 *Betula verrucosa* (*Betula pendula*) (European white birch)  
 Bet v 1.0113 Q96370 *Betula verrucosa* (*Betula pendula*) (European white birch)  
 Bet v 1.0114 Q96371 *Betula verrucosa* (*Betula pendula*) (European white birch)  
 Bet v 1.0201 P45431 *Betula verrucosa* (*Betula pendula*) (European white birch)  
 Bet v 1.0202 P43176 *Betula verrucosa* (*Betula pendula*) (European white birch)  
 Bet v 1.0203 P43184 *Betula verrucosa* (*Betula pendula*) (European white birch)  
 Bet v 1.0204 P43186 *Betula verrucosa* (*Betula pendula*) (European white birch)  
 Bet v 2.0101 P25816 *Betula verrucosa* (*Betula pendula*) (European white birch)  
 Bet v 3.0101 P43187 *Betula verrucosa* (*Betula pendula*) (European white birch)  
 Bet v 4.0101 Q39419 *Betula verrucosa* (*Betula pendula*) (European white birch)  
 Bet v 6.0101 Q65002 *Betula verrucosa* (*Betula pendula*) (European white birch)  
 Bet v 6.0102 Q9FUW6 *Betula verrucosa* (*Betula pendula*) (European white birch)  
 Bet v 7.0101 P81531 *Betula verrucosa* (*Betula pendula*) (European white birch)  
 Beta v 1.0101 P85983 *Beta vulgaris* (Sugar beet)  
 Beta v 2.0101 P85984 *Beta vulgaris* (Sugar beet)  
 Bla g 1.0101 Q9UAM5 *Blattella germanica* (German cockroach)  
 Bla g 1.0201 Q96522 *Blattella germanica* (German cockroach)  
 Bla g 2.0101 P54958 *Blattella germanica* (German cockroach)  
 Bla g 4.0101 P54962 *Blattella germanica* (German cockroach)  
 Bla g 5.0101 Q18598 *Blattella germanica* (German cockroach)  
 Bla g 6.0101 Q1A7B3 *Blattella germanica* (German cockroach)  
 Bla g 6.0201 Q1A7B2 *Blattella germanica* (German cockroach)  
 Bla g 6.0301 Q1A7B1 *Blattella germanica* (German cockroach)  
 Bla g 7.0101 Q9NG56 *Blattella germanica* (German cockroach)  
 Bla g 8.0101 AOERA8 *Blattella germanica* (German cockroach)  
 Blo t 1.0101 Q95PJ4 *Blomia tropicalis* (Mite)  
 Blo t 10.0101 A7XZI4 *Blomia tropicalis* (Mite)  
 Blo t 11.0101 Q8MUF6 *Blomia tropicalis* (Mite)  
 Blo t 12.0101 Q17282 *Blomia tropicalis* (Mite)  
 Blo t 13.0101 Q17284 *Blomia tropicalis* (Mite)  
 Blo t 2.0101 Q1M2P1 *Blomia tropicalis* (Mite)  
 Blo t 2.0102 Q1M2P2 *Blomia tropicalis* (Mite)  
 Blo t 2.0103 Q1M2P3 *Blomia tropicalis* (Mite)  
 Blo t 21.0101 A7IZE9 *Blomia tropicalis* (Mite)  
 Blo t 3.0101 Q8I916 *Blomia tropicalis* (Mite)  
 Blo t 5.0101 Q96870 *Blomia tropicalis* (Mite)  
 Bom p 1.0101 Q7M4I6 *Bombus pennsylvanicus* (Bumble bee)  
 Bom p 4.0101 Q7M4I3 *Bombus pennsylvanicus* (Bumble bee)  
 Bom t 1.0101 P82971 *Bombus terrestris* (Bumble bee)  
 Bos d 2.0101 Q28133 *Bos domesticus* (domestic cattle)

Bos d 2.0102 Q28133 *Bos domesticus* (domestic cattle)  
 Bos d 2.0103 Q28133 *Bos domesticus* (domestic cattle)  
 Bos d 3.0101 Q28050 *Bos domesticus* (domestic cattle)  
 Bos d 4.0101 P00711 *Bos domesticus* (domestic cattle)  
 Bos d 5.0101 P02754 *Bos domesticus* (domestic cattle)  
 Bos d 6.0101 P02769 *Bos domesticus* (domestic cattle)  
 Bra j 1.0101 P80207 *Brassica juncea* (Oriental mustard)  
 Bra n 1.0101 P80208 *Brassica napus* (Rapeseed)  
 Bra r 1.0101 Q42473 *Brassica rapa* (Turnip)  
 Bra r 2.0101 P81729 *Brassica rapa* (Turnip)  
 Can f 1.0101 O18873 *Canis familiaris* (dog)  
 Can f 2.0101 O18874 *Canis familiaris* (dog)  
 Can f 3.0101 P49822 *Canis familiaris* (dog)  
 Can f 5.0101 P09582 *Canis familiaris* (dog)  
 Cand a 1.0101 P43067 *Candida albicans* (Yeast)  
 Cand a 3.0101 Q6YK78 *Candida albicans* (Yeast)  
 Cand b 2.0101 P14292 *Candida boidinii* (Yeast)  
 Cap a 1w.0101 Q9ARGO *Capsicum annuum* (Bell pepper)  
 Cap a 2.0101 Q93YI9 *Capsicum annuum* (Bell pepper)  
 Car b 1.0101 P38949 *Carpinus betulus* (Hornbeam)  
 Car b 1.0102 P38949 *Carpinus betulus* (Hornbeam)  
 Car b 1.0103 Q96377 *Carpinus betulus* (Hornbeam)  
 Car b 1.0104 Q96378 *Carpinus betulus* (Hornbeam)  
 Car b 1.0105 Q96379 *Carpinus betulus* (Hornbeam)  
 Car b 1.0106 Q96503 *Carpinus betulus* (Hornbeam)  
 Car b 1.0107 Q96501 *Carpinus betulus* (Hornbeam)  
 Car b 1.0108 Q96380 *Carpinus betulus* (Hornbeam)  
 Car b 1.0109 B6RQR6 *Carpinus betulus* (Hornbeam)  
 Car b 1.0110 B6RQR7 *Carpinus betulus* (Hornbeam)  
 Car b 1.0111 B6RQR8 *Carpinus betulus* (Hornbeam)  
 Car b 1.0112 B6RQR9 *Carpinus betulus* (Hornbeam)  
 Car b 1.0113 B6RQ50 *Carpinus betulus* (Hornbeam)  
 Car b 1.0201 P38950 *Carpinus betulus* (Hornbeam)  
 Car b 1.0301 Q96381 *Carpinus betulus* (Hornbeam)  
 Car b 1.0302 Q96382 *Carpinus betulus* (Hornbeam)  
 Car i 1.0101 Q84XA9 *Carya illinoensis* (Pecan)  
 Car i 4.0101 B5KVH4 *Carya illinoensis* (Pecan)  
 Cas s 1.0101 B7TWE3 *Castanea sativa* (Chestnut)  
 Cat r 1.0101 Q39613 *Catharanthus roseus* (Rosy periwinkle)  
 Cav p 1.0101 P83507 *Cavia porcellus* (guinea pig)  
 Cav p 2.0101 F0UZ11 *Cavia porcellus* (guinea pig)  
 Cav p 3.0101 F0UZ12 *Cavia porcellus* (guinea pig)  
 Cha f 1.0101 Q9N2R3 *Charybdis feriatus* (Crab)  
 Cha o 1.0101 Q96385 *Chamaecyparis obtusa* (Japanese cypress)  
 Cha o 2.0101 Q7M1E7 *Chamaecyparis obtusa* (Japanese cypress)  
 Che a 1.0101 Q8LGR0 *Chenopodium album* (Pigweed)  
 Che a 2.0101 Q84V37 *Chenopodium album* (Pigweed)  
 Che a 3.0101 Q84V36 *Chenopodium album* (Pigweed)  
 Chi k 10.0101 O96764 *Chironomus kiiensis* (Midge)  
 Chi t 1.0101 P02229 *Chironomus thummi thummi* (Midge)  
 Chi t 1.0201 P02230 *Chironomus thummi thummi* (Midge)  
 Chi t 2.0101 P02221 *Chironomus thummi thummi* (Midge)  
 Chi t 2.0102 P02221 *Chironomus thummi thummi* (Midge)  
 Chi t 3.0101 P02222 *Chironomus thummi thummi* (Midge)  
 Chi t 3.0201 P02224 *Chironomus thummi thummi* (Midge)  
 Chi t 3.0301 P02226 *Chironomus thummi thummi* (Midge)  
 Chi t 3.0401 P02223 *Chironomus thummi thummi* (Midge)  
 Chi t 3.0501 P12548 *Chironomus thummi thummi* (Midge)  
 Chi t 3.0601 P84296 *Chironomus thummi thummi* (Midge)  
 Chi t 3.0701 P84298 *Chironomus thummi thummi* (Midge)  
 Chi t 3.0702 P12549 *Chironomus thummi thummi* (Midge)  
 Chi t 3.0801 P12550 *Chironomus thummi thummi* (Midge)  
 Chi t 3.0901 P02227 *Chironomus thummi thummi* (Midge)  
 Chi t 4.0101 P02231 *Chironomus thummi thummi* (Midge)  
 Chi t 9.0101 P02228 *Chironomus thummi thummi* (Midge)  
 Cit l 3.0101 P84160 *Citrus limon* (Lemon)  
 Cit s 1.0101 P84159 *Citrus sinensis* (Sweet orange)  
 Cit s 2.0101 P84177 *Citrus sinensis* (Sweet orange)  
 Cit s 3.0102 Q6EV47 *Citrus sinensis* (Sweet orange)

Cla c 9.0101 B0L807 Cladosporium cladosporioides  
 Cla h 10.0101 P40108 Cladosporium herbarum  
 Cla h 12.0101 P50344 Cladosporium herbarum  
 Cla h 5.0101 P42039 Cladosporium herbarum  
 Cla h 6.0101 P42040 Cladosporium herbarum  
 Cla h 7.0101 P42059 Cladosporium herbarum  
 Cla h 8.0101 P0C0Y5 Cladosporium herbarum  
 Cla h 9.0101 B7ZK61 Cladosporium herbarum  
 Clu h 1.0101 C6GKU6 Clupea harengus (Atlantic herring)  
 Clu h 1.0201 C6GKU7 Clupea harengus (Atlantic herring)  
 Clu h 1.0301 C6GKU8 Clupea harengus (Atlantic herring)  
 Cop c 1.0101 Q9Y7G3 Coprinus comatus (Shaggy mane)  
 Cop c 2.0101 Q9UW02 Coprinus comatus (Shaggy mane)  
 Cop c 3.0101 Q9UW01 Coprinus comatus (Shaggy mane)  
 Cop c 5.0101 Q9UW00 Coprinus comatus (Shaggy mane)  
 Cop c 7.0101 Q9UVZ9 Coprinus comatus (Shaggy mane)  
 Cor a 1.0101 Q08407 Corylus avellana (Hazel)  
 Cor a 1.0102 Q08407 Corylus avellana (Hazel)  
 Cor a 1.0103 Q08407 Corylus avellana (Hazel)  
 Cor a 1.0104 Q08407 Corylus avellana (Hazel)  
 Cor a 1.0201 Q39453 Corylus avellana (Hazel)  
 Cor a 1.0301 Q39454 Corylus avellana (Hazel)  
 Cor a 1.0401 Q9SWR4 Corylus avellana (Hazel)  
 Cor a 1.0402 Q9FPK4 Corylus avellana (Hazel)  
 Cor a 1.0403 Q9FPK3 Corylus avellana (Hazel)  
 Cor a 1.0404 Q9FPK2 Corylus avellana (Hazel)  
 Cor a 10.0101 Q9FSY7 Corylus avellana (Hazel)  
 Cor a 11.0101 Q8S4P9 Corylus avellana (Hazel)  
 Cor a 12.0101 Q84T21 Corylus avellana (Hazel)  
 Cor a 13.0101 Q84T91 Corylus avellana (Hazel)  
 Cor a 14.0101 D0PWG2 Corylus avellana (Hazel)  
 Cor a 2.0101 Q9AXH5 Corylus avellana (Hazel)  
 Cor a 2.0102 Q9AXH4 Corylus avellana (Hazel)  
 Cor a 8.0101 Q9ATH2 Corylus avellana (Hazel)  
 Cor a 9.0101 Q8W1C2 Corylus avellana (Hazel)  
 Cra c 1.0101 D7F1J4 Crangon crangon (North Sea shrimp)  
 Cra c 2.0101 D7F1J5 Crangon crangon (North Sea shrimp)  
 Cra c 4.0101 D7F1P9 Crangon crangon (North Sea shrimp)  
 Cra c 5.0101 D7F1Q1 Crangon crangon (North Sea shrimp)  
 Cra c 6.0101 D7F1Q2 Crangon crangon (North Sea shrimp)  
 Cra c 8.0101 D7F1Q0 Crangon crangon (North Sea shrimp)  
 Cro s 1.0101 Q29W25 Crocus sativus (Saffron crocus)  
 Cry j 1.0101 P18632 Cryptomeria japonica (Sugi)  
 Cry j 1.0102 P18632 Cryptomeria japonica (Sugi)  
 Cry j 1.0103 P18632 Cryptomeria japonica (Sugi)  
 Cry j 1.0103 Q8RUR1 Cryptomeria japonica (Sugi)  
 Cry j 1.0103 Q8RUR1 Cryptomeria japonica (Sugi)  
 Cry j 2.0101 P43212 Cryptomeria japonica (Sugi)  
 Cte f 1.0101 Q94424 Ctenocephalides felis felis (Cat flea)  
 Cte f 2.0101 Q9NH66 Ctenocephalides felis felis (Cat flea)  
 Cuc m 1.0101 Q39547 Cucumis melo (Muskmelon)  
 Cuc m 2.0101 Q5FX67 Cucumis melo (Muskmelon)  
 Cuc m 3.0101 P83834 Cucumis melo (Muskmelon)  
 Cup a 1.0101 Q9SCG9 Cupressus arizonica (Cypress)  
 Cup s 1.0101 Q9M4S6 Cupressus sempervirens (Common cypress)  
 Cup s 1.0102 Q9M4S5 Cupressus sempervirens (Common cypress)  
 Cup s 1.0103 Q9M4S4 Cupressus sempervirens (Common cypress)  
 Cup s 1.0104 Q9M4S3 Cupressus sempervirens (Common cypress)  
 Cup s 1.0105 Q9M4S2 Cupressus sempervirens (Common cypress)  
 Cup s 3.0101 Q69CS2 Cupressus sempervirens (Common cypress)  
 Cup s 3.0102 Q69CS3 Cupressus sempervirens (Common cypress)  
 Cup s 3.0103 Q69CS2 Cupressus sempervirens (Common cypress)  
 Cur l 2.0101 Q96VP4 Curvularia lunata (Synonym: Cochliobolus lunatus)  
 Cur l 3.0101 Q96VP3 Curvularia lunata (Synonym: Cochliobolus lunatus)  
 Cyn d 1.0101 004701 Cynodon dactylon (Bermuda grass)  
 Cyn d 1.0201 Q947S7 Cynodon dactylon (Bermuda grass)  
 Cyn d 1.0202 Q947S6 Cynodon dactylon (Bermuda grass)  
 Cyn d 1.0203 Q947S4 Cynodon dactylon (Bermuda grass)  
 Cyn d 1.0204 Q9FVMO Cynodon dactylon (Bermuda grass)

Cyn d 12.0101 004725 *Cynodon dactylon* (Bermuda grass)  
Cyn d 15.0101 Q7XYF2 *Cynodon dactylon* (Bermuda grass)  
Cyn d 23.0101 Q7XYF3 *Cynodon dactylon* (Bermuda grass)  
Cyn d 24.0101 Q647J6 *Cynodon dactylon* (Bermuda grass)  
Cyn d 7.0101 P94092 *Cynodon dactylon* (Bermuda grass)  
Dac g 1.0101 Q7M1X8 *Dactylis glomerata* (Orchard grass)  
Dac g 2.0101 Q41183 *Dactylis glomerata* (Orchard grass)  
Dac g 3.0101 P93124 *Dactylis glomerata* (Orchard grass)  
Dac g 4.0101 P82946 *Dactylis glomerata* (Orchard grass)  
Dau c 1.0101 004298 *Daucus carota* (Carrot)  
Dau c 1.0102 004298 *Daucus carota* (Carrot)  
Dau c 1.0103 004298 *Daucus carota* (Carrot)  
Dau c 1.0104 004298 *Daucus carota* (Carrot)  
Dau c 1.0105 004298 *Daucus carota* (Carrot)  
Dau c 1.0201 Q8SAE7 *Daucus carota* (Carrot)  
Dau c 4.0101 Q8SAE6 *Daucus carota* (Carrot)  
Der f 1.0101 Q58A71 *Dermatophagoides farinae* (American house dust mite)  
Der f 1.0102 Q3HWZ4 *Dermatophagoides farinae* (American house dust mite)  
Der f 1.0103 Q3HWZ4 *Dermatophagoides farinae* (American house dust mite)  
Der f 1.0104 Q3HWZ4 *Dermatophagoides farinae* (American house dust mite)  
Der f 1.0105 Q3HWZ4 *Dermatophagoides farinae* (American house dust mite)  
Der f 1.0106 P16311 *Dermatophagoides farinae* (American house dust mite)  
Der f 1.0108 A1YW11 *Dermatophagoides farinae* (American house dust mite)  
Der f 1.0109 A1YW12 *Dermatophagoides farinae* (American house dust mite)  
Der f 1.0110 A1YW13 *Dermatophagoides farinae* (American house dust mite)  
Der f 10.0101 Q23939 *Dermatophagoides farinae* (American house dust mite)  
Der f 11.0101 Q967Z0 *Dermatophagoides farinae* (American house dust mite)  
Der f 13.0101 Q1M2P5 *Dermatophagoides farinae* (American house dust mite)  
Der f 14.0101 Q94507 *Dermatophagoides farinae* (American house dust mite)  
Der f 15.0101 Q9U6R7 *Dermatophagoides farinae* (American house dust mite)  
Der f 16.0101 Q8MVU3 *Dermatophagoides farinae* (American house dust mite)  
Der f 18.0101 Q86R84 *Dermatophagoides farinae* (American house dust mite)  
Der f 2.0101 Q00855 *Dermatophagoides farinae* (American house dust mite)  
Der f 2.0102 Q00855 *Dermatophagoides farinae* (American house dust mite)  
Der f 2.0103 Q00855 *Dermatophagoides farinae* (American house dust mite)  
Der f 2.0105 Q8WQK5 *Dermatophagoides farinae* (American house dust mite)  
Der f 2.0106 Q5TIW2 *Dermatophagoides farinae* (American house dust mite)  
Der f 2.0107 Q5TIW1 *Dermatophagoides farinae* (American house dust mite)  
Der f 2.0108 Q5TIW0 *Dermatophagoides farinae* (American house dust mite)  
Der f 2.0109 Q3HWZ2 *Dermatophagoides farinae* (American house dust mite)  
Der f 2.0112 A1KXH0 *Dermatophagoides farinae* (American house dust mite)  
Der f 2.0116 A3F5F1 *Dermatophagoides farinae* (American house dust mite)  
Der f 22.0101 A5X5X4 *Dermatophagoides farinae* (American house dust mite)  
Der f 3.0101 P49275 *Dermatophagoides farinae* (American house dust mite)  
Der f 6.0101 P49276 *Dermatophagoides farinae* (American house dust mite)  
Der f 7.0101 Q26456 *Dermatophagoides farinae* (American house dust mite)  
Der m 1.0101 P16312 *Dermatophagoides microceras* (House dust mite)  
Der p 1.0101 P08176 *Dermatophagoides pteronyssinus* (European house dust mite)  
Der p 1.0102 P08176 *Dermatophagoides pteronyssinus* (European house dust mite)  
Der p 1.0103 P08176 *Dermatophagoides pteronyssinus* (European house dust mite)  
Der p 1.0104 P08176 *Dermatophagoides pteronyssinus* (European house dust mite)  
Der p 1.0105 P08176 *Dermatophagoides pteronyssinus* (European house dust mite)  
Der p 1.0106 P08176 *Dermatophagoides pteronyssinus* (European house dust mite)  
Der p 1.0107 P08176 *Dermatophagoides pteronyssinus* (European house dust mite)  
Der p 1.0108 P08176 *Dermatophagoides pteronyssinus* (European house dust mite)  
Der p 1.0109 P08176 *Dermatophagoides pteronyssinus* (European house dust mite)  
Der p 1.0110 P08176 *Dermatophagoides pteronyssinus* (European house dust mite)  
Der p 1.0111 P08176 *Dermatophagoides pteronyssinus* (European house dust mite)  
Der p 1.0112 P08176 *Dermatophagoides pteronyssinus* (European house dust mite)  
Der p 1.0113 Q3HWZ5 *Dermatophagoides pteronyssinus* (European house dust mite)  
Der p 1.0114 Q3HWZ5 *Dermatophagoides pteronyssinus* (European house dust mite)  
Der p 1.0115 Q3HWZ5 *Dermatophagoides pteronyssinus* (European house dust mite)  
Der p 1.0116 Q3HWZ5 *Dermatophagoides pteronyssinus* (European house dust mite)  
Der p 1.0117 Q3HWZ5 *Dermatophagoides pteronyssinus* (European house dust mite)  
Der p 1.0118 Q3HWZ5 *Dermatophagoides pteronyssinus* (European house dust mite)  
Der p 1.0119 Q3HWZ5 *Dermatophagoides pteronyssinus* (European house dust mite)  
Der p 1.0120 Q3HWZ5 *Dermatophagoides pteronyssinus* (European house dust mite)  
Der p 1.0121 Q3HWZ5 *Dermatophagoides pteronyssinus* (European house dust mite)  
Der p 1.0122 Q3HWZ5 *Dermatophagoides pteronyssinus* (European house dust mite)

Der p 1.0123 Q3HWZ5 Dermatophagoides pteronyssinus (European house dust mite)  
Der p 1.0124 C7T6L6 Dermatophagoides pteronyssinus (European house dust mite)  
Der p 10.0101 D18416 Dermatophagoides pteronyssinus (European house dust mite)  
Der p 11.0101 Q6Y2F9 Dermatophagoides pteronyssinus (European house dust mite)  
Der p 14.0101 Q8NONO Dermatophagoides pteronyssinus (European house dust mite)  
Der p 2.0101 P49278 Dermatophagoides pteronyssinus (European house dust mite)  
Der p 2.0102 P49278 Dermatophagoides pteronyssinus (European house dust mite)  
Der p 2.0103 P49278 Dermatophagoides pteronyssinus (European house dust mite)  
Der p 2.0104 P49278 Dermatophagoides pteronyssinus (European house dust mite)  
Der p 2.0105 P49278 Dermatophagoides pteronyssinus (European house dust mite)  
Der p 2.0106 P49278 Dermatophagoides pteronyssinus (European house dust mite)  
Der p 2.0107 P49278 Dermatophagoides pteronyssinus (European house dust mite)  
Der p 2.0108 P49278 Dermatophagoides pteronyssinus (European house dust mite)  
Der p 2.0110 C7T6L5 Dermatophagoides pteronyssinus (European house dust mite)  
Der p 2.0114 Q1H8P8 Dermatophagoides pteronyssinus (European house dust mite)  
Der p 2.0115 C7T6L5 Dermatophagoides pteronyssinus (European house dust mite)  
Der p 21.0101 Q2L7C5 Dermatophagoides pteronyssinus (European house dust mite)  
Der p 3.0101 P39675 Dermatophagoides pteronyssinus (European house dust mite)  
Der p 4.0101 Q9Y197 Dermatophagoides pteronyssinus (European house dust mite)  
Der p 5.0101 P14004 Dermatophagoides pteronyssinus (European house dust mite)  
Der p 5.0102 P14004 Dermatophagoides pteronyssinus (European house dust mite)  
Der p 6.0101 P49277 Dermatophagoides pteronyssinus (European house dust mite)  
Der p 7.0101 P49273 Dermatophagoides pteronyssinus (European house dust mite)  
Der p 8.0101 P46419 Dermatophagoides pteronyssinus (European house dust mite)  
Der p 9.0101 Q7Z163 Dermatophagoides pteronyssinus (European house dust mite)  
Der p 9.0102 Q8MWR4 Dermatophagoides pteronyssinus (European house dust mite)  
Dol a 5.0101 Q05108 Dolichovespula arenaria (Yellow hornet)  
Dol m 1.0101 Q06478 Dolichovespula maculata (White face hornet)  
Dol m 1.02 P53357 Dolichovespula maculata (White face hornet)  
Dol m 2.0101 P49371 Dolichovespula maculata (White face hornet)  
Dol m 5.0101 P10736 Dolichovespula maculata (White face hornet)  
Dol m 5.02 P10737 Dolichovespula maculata (White face hornet)  
Epi p 1.0101 P83340 Epicoccum purpurascens (Soil fungus)  
Equ c 1.0101 Q95182 Equus caballus (domestic horse)  
Equ c 2.0101 P81216 Equus caballus (domestic horse)  
Equ c 2.0102 P81217 Equus caballus (domestic horse)  
Equ c 3.0101 P35747 Equus caballus (domestic horse)  
Equ c 4.0101 P82615 Equus caballus (domestic horse)  
Eur m 1.0101 P25780 Euroglyphus maynei (House dust mite)  
Eur m 1.0102 P25780 Euroglyphus maynei (House dust mite)  
Eur m 14.0101 Q9U785 Euroglyphus maynei (House dust mite)  
Eur m 2.0101 Q9TZ22 Euroglyphus maynei (House dust mite)  
Eur m 2.0102 Q9TZ22 Euroglyphus maynei (House dust mite)  
Eur m 3.0101 Q9Y370 Euroglyphus maynei (House dust mite)  
Eur m 4.0101 Q9Y196 Euroglyphus maynei (House dust mite)  
Fel d 1.0101 P30438 Felis domesticus (cat)  
Fel d 2.0101 P49064 Felis domesticus (cat)  
Fel d 3.0101 Q8WNR9 Felis domesticus (cat)  
Fel d 4.0101 Q5VFH6 Felis domesticus (cat)  
For t 1.0101 B2ZPG6 Forcipomyia taiwana (Biting midge)  
For t 2.0101 B2ZPG7 Forcipomyia taiwana (Biting midge)  
Fra a 1.0101 Q5ULZ4 Fragaria ananassa (Strawberry)  
Fra a 3.0101 Q8VX12 Fragaria ananassa (Strawberry)  
Fra a 3.0202 Q4PLT6 Fragaria ananassa (Strawberry)  
Fra e 1.0101 Q7XAV4 Fraxinus excelsior (Ash)  
Fra e 1.0102 Q5EXJ6 Fraxinus excelsior (Ash)  
Fra e 1.0201 Q6U740 Fraxinus excelsior (Ash)  
Fus c 1.0101 Q8TFM9 Fusarium culmorum (N.A.)  
Fus c 2.0101 Q8TFM8 Fusarium culmorum (N.A.)  
Gad c 1.0101 P02622 Gadus callarias (Baltic cod)  
Gal d 1.0101 P01005 Gallus domesticus (chicken)  
Gal d 2.0101 P01012 Gallus domesticus (chicken)  
Gal d 3.0101 P02789 Gallus domesticus (chicken)  
Gal d 4.0101 P00698 Gallus domesticus (chicken)  
Gal d 5.0101 P19121 Gallus domesticus (chicken)  
Gal d 6.0101 P87498 Gallus domesticus (chicken)  
Gly d 2.0101 Q9U5P7 Glycyphagus domesticus (Storage mite)  
Gly d 2.0201 Q9NFK4 Glycyphagus domesticus (Storage mite)  
Gly m 1.0101 Q9S8F3 Glycine max (Soybean)

Gly m 1.0102 Q9S8F2 Glycine max (Soybean)  
 Gly m 3.0101 065809 Glycine max (Soybean)  
 Gly m 3.0102 065810 Glycine max (Soybean)  
 Gly m 4.0101 P26987 Glycine max (Soybean)  
 Gly m 5.0101 022120 Glycine max (Soybean)  
 Gly m 5.0201 Q9FZP9 Glycine max (Soybean)  
 Gly m 5.0301 P25974 Glycine max (Soybean)  
 Gly m 5.0302 P25974 Glycine max (Soybean)  
 Gly m 6.0101 P04776 Glycine max (Soybean)  
 Gly m 6.0201 P04405 Glycine max (Soybean)  
 Gly m 6.0301 P11828 Glycine max (Soybean)  
 Gly m 6.0401 Q9SB11 Glycine max (Soybean)  
 Gly m 6.0501 Q7GC77 Glycine max (Soybean)  
 Hel a 2.0101 081982 Helianthus annuus (Sunflower)  
 Hel a 3.0101 Q7X9Q5 Helianthus annuus (Sunflower)  
 Hel as 1.0101 097192 Helix aspersa (Brown garden snail)  
 Hev b 1.0101 P15252 Hevea brasiliensis (Para rubber tree (latex))  
 Hev b 10.0101 P35017 Hevea brasiliensis (Para rubber tree (latex))  
 Hev b 10.0102 Q9STB5 Hevea brasiliensis (Para rubber tree (latex))  
 Hev b 10.0103 Q9FSJ2 Hevea brasiliensis (Para rubber tree (latex))  
 Hev b 11.0101 Q949H3 Hevea brasiliensis (Para rubber tree (latex))  
 Hev b 11.0102 Q8GUD7 Hevea brasiliensis (Para rubber tree (latex))  
 Hev b 12.0101 Q8RYA8 Hevea brasiliensis (Para rubber tree (latex))  
 Hev b 13.0101 Q7Y1X1 Hevea brasiliensis (Para rubber tree (latex))  
 Hev b 2.0101 P52407 Hevea brasiliensis (Para rubber tree (latex))  
 Hev b 3.0101 082803 Hevea brasiliensis (Para rubber tree (latex))  
 Hev b 4.0101 Q6T4P0 Hevea brasiliensis (Para rubber tree (latex))  
 Hev b 5.0101 Q39967 Hevea brasiliensis (Para rubber tree (latex))  
 Hev b 6.01 P02877 Hevea brasiliensis (Para rubber tree (latex))  
 Hev b 6.02 P02877 Hevea brasiliensis (Para rubber tree (latex))  
 Hev b 6.03 P02877 Hevea brasiliensis (Para rubber tree (latex))  
 Hev b 7.01 004008 Hevea brasiliensis (Para rubber tree (latex))  
 Hev b 7.02 065811 Hevea brasiliensis (Para rubber tree (latex))  
 Hev b 8.0101 065812 Hevea brasiliensis (Para rubber tree (latex))  
 Hev b 8.0102 Q9STB6 Hevea brasiliensis (Para rubber tree (latex))  
 Hev b 8.0201 Q9M7N0 Hevea brasiliensis (Para rubber tree (latex))  
 Hev b 8.0202 Q9M7M9 Hevea brasiliensis (Para rubber tree (latex))  
 Hev b 8.0203 Q9M7M8 Hevea brasiliensis (Para rubber tree (latex))  
 Hev b 8.0204 Q9LEI8 Hevea brasiliensis (Para rubber tree (latex))  
 Hev b 9.0101 Q9LEJ0 Hevea brasiliensis (Para rubber tree (latex))  
 Hol l 1.0101 P43216 Holcus lanatus (Velvet grass)  
 Hol l 1.0102 P43216 Holcus lanatus (Velvet grass)  
 Hol l 5.0101 023972 Holcus lanatus (Velvet grass)  
 Hol l 5.0201 023971 Holcus lanatus (Velvet grass)  
 Hom a 1.0101 044119 Homarus americanus (American lobster)  
 Hom a 1.0102 044119 Homarus americanus (American lobster)  
 Hom a 6.0101 P29291 Homarus americanus (American lobster)  
 Hom s 1.0101 043290 Homo sapiens (human autoallergens)  
 Hom s 2.0101 Q13765 Homo sapiens (human autoallergens)  
 Hom s 3.0101 Q13845 Homo sapiens (human autoallergens)  
 Hom s 4.0101 075785 Homo sapiens (human autoallergens)  
 Hom s 5.0101 P02538 Homo sapiens (human autoallergens)  
 Hor v 12.0101 P52184 Hordeum vulgare (Barley)  
 Hor v 15.0101 P16968 Hordeum vulgare (Barley)  
 Hor v 20.0101 P80198 Hordeum vulgare (Barley)  
 Hor v 5.0101 004828 Hordeum vulgare (Barley)  
 Hum j 1.0101 Q7XBE3 Humulus japonicus (Japanese hop)  
 Jug n 1.0101 Q7Y1C2 Juglans nigra (Black walnut)  
 Jug n 2.0101 Q7Y1C1 Juglans nigra (Black walnut)  
 Jug r 1.0101 P93198 Juglans regia (English walnut)  
 Jug r 2.0101 Q9SEW4 Juglans regia (English walnut)  
 Jug r 4.0101 Q2TPW5 Juglans regia (English walnut)  
 Jun a 1.010101 P81294 Juniperus ashei (Mountain cedar)  
 Jun a 1.010102 P81294 Juniperus ashei (Mountain cedar)  
 Jun a 2.0101 Q9FY19 Juniperus ashei (Mountain cedar)  
 Jun a 3.0101 P81295 Juniperus ashei (Mountain cedar)  
 Jun o 4.0101 064943 Juniperus oxycedrus (Prickly juniper)  
 Jun v 1.0101 Q9LLT2 Juniperus virginiana (Eastern red cedar)  
 Jun v 1.0102 Q9LLT1 Juniperus virginiana (Eastern red cedar)

Jun v 3.010101 Q9LD79 *Juniperus virginiana* (Eastern red cedar)  
 Jun v 3.010102 Q9LD79 *Juniperus virginiana* (Eastern red cedar)  
 Len c 1.0101 Q84UI1 *Lens culinaris* (Lentil)  
 Len c 1.0102 Q84UI0 *Lens culinaris* (Lentil)  
 Lep d 10.0101 Q9NFZ4 *Lepidoglyphus destructor* (Storage mite)  
 Lep d 13.0101 Q9U5P1 *Lepidoglyphus destructor* (Storage mite)  
 Lep d 2.0101 P80384 *Lepidoglyphus destructor* (Storage mite)  
 Lep d 2.0102 P80384 *Lepidoglyphus destructor* (Storage mite)  
 Lep d 2.0201 P80384 *Lepidoglyphus destructor* (Storage mite)  
 Lep d 2.0202 P80384 *Lepidoglyphus destructor* (Storage mite)  
 Lep d 5.0101 Q9U5P2 *Lepidoglyphus destructor* (Storage mite)  
 Lep d 5.0102 Q1M2N1 *Lepidoglyphus destructor* (Storage mite)  
 Lep d 5.0103 Q1M2N0 *Lepidoglyphus destructor* (Storage mite)  
 Lep d 7.0101 Q9U1G2 *Lepidoglyphus destructor* (Storage mite)  
 Lep s 1.0101 Q8T380 *Lepisma saccharina* (Silverfish)  
 Lep w 1.0101 B5WX08 *Lepidorhombus whiffiagonis* (Megrim, Whiff, Gallo)  
 Lig v 1.0101 O82015 *Ligustrum vulgare* (Privet)  
 Lig v 1.0102 O82015 *Ligustrum vulgare* (Privet)  
 Lit c 1.0101 Q941H7 *Litchi chinensis* (Litchi)  
 Lit v 1.0101 B4YAH6 *Litopenaeus vannamei* (White shrimp)  
 Lit v 2.0101 Q004B5 *Litopenaeus vannamei* (White shrimp)  
 Lit v 3.0101 B7SNI3 *Litopenaeus vannamei* (White shrimp)  
 Lit v 4.0101 C7A639 *Litopenaeus vannamei* (White shrimp)  
 Lol p 1.0101 P14946 *Lolium perenne* (Rye grass)  
 Lol p 1.0102 P14946 *Lolium perenne* (Rye grass)  
 Lol p 1.0103 Q9SC98 *Lolium perenne* (Rye grass)  
 Lol p 11.0101 Q7M1X5 *Lolium perenne* (Rye grass)  
 Lol p 2.0101 P14947 *Lolium perenne* (Rye grass)  
 Lol p 3.0101 P14948 *Lolium perenne* (Rye grass)  
 Lol p 4.0101 Q5TIW3 *Lolium perenne* (Rye grass)  
 Lol p 5.0101 Q40237 *Lolium perenne* (Rye grass)  
 Lol p 5.0102 Q40240 *Lolium perenne* (Rye grass)  
 Lup an 1.0101 B8Q5G0 *Lupinus angustifolius* (Narrow-leaved blue lupin)  
 Sola l 1.0101 Q93YG7 *Solanum lycopersicum* (*Lycopersicon esculentum*) (Tomato)  
 Sola l 2.0101 Q54YQ0 *Solanum lycopersicum* (*Lycopersicon esculentum*) (Tomato)  
 Sola l 2.0201 Q8RVW4 *Solanum lycopersicum* (*Lycopersicon esculentum*) (Tomato)  
 Sola l 3.0101 P93224 *Solanum lycopersicum* (*Lycopersicon esculentum*) (Tomato)  
 Mal d 1.0101 P43211 *Malus domestica* (Apple)  
 Mal d 1.0102 P43211 *Malus domestica* (Apple)  
 Mal d 1.0103 Q9SYV2 *Malus domestica* (Apple)  
 Mal d 1.0104 Q9SYV5 *Malus domestica* (Apple)  
 Mal d 1.0105 Q9SYV6 *Malus domestica* (Apple)  
 Mal d 1.0106 Q9SYV7 *Malus domestica* (Apple)  
 Mal d 1.0107 Q9SYV8 *Malus domestica* (Apple)  
 Mal d 1.0108 Q9SYW3 *Malus domestica* (Apple)  
 Mal d 1.0109 Q941P6 *Malus domestica* (Apple)  
 Mal d 1.0201 Q40280 *Malus domestica* (Apple)  
 Mal d 1.0202 Q9S7M5 *Malus domestica* (Apple)  
 Mal d 1.0203 Q9SYV3 *Malus domestica* (Apple)  
 Mal d 1.0204 Q9SYV4 *Malus domestica* (Apple)  
 Mal d 1.0205 Q9SYV9 *Malus domestica* (Apple)  
 Mal d 1.0206 Q40280 *Malus domestica* (Apple)  
 Mal d 1.0207 Q941P5 *Malus domestica* (Apple)  
 Mal d 1.0208 Q8L6K9 *Malus domestica* (Apple)  
 Mal d 1.0301 Q43549 *Malus domestica* (Apple)  
 Mal d 1.0302 Q941P8 *Malus domestica* (Apple)  
 Mal d 1.0303 Q941P7 *Malus domestica* (Apple)  
 Mal d 1.0304 Q84LA7 *Malus domestica* (Apple)  
 Mal d 1.0401 Q43550 *Malus domestica* (Apple)  
 Mal d 1.0402 Q43551 *Malus domestica* (Apple)  
 Mal d 1.0403 Q43552 *Malus domestica* (Apple)  
 Mal d 2.0101 Q9FSG7 *Malus domestica* (Apple)  
 Mal d 4.0101 Q9XF42 *Malus domestica* (Apple)  
 Mal d 4.0102 Q84RR5 *Malus domestica* (Apple)  
 Mal d 4.0201 Q9XF41 *Malus domestica* (Apple)  
 Mal d 4.0202 Q84RR6 *Malus domestica* (Apple)  
 Mal d 4.0301 Q9XF40 *Malus domestica* (Apple)  
 Mal d 4.0302 Q84RR7 *Malus domestica* (Apple)  
 Mala f 2.0101 P56577 *Malassezia furfur* (Pityriasis versicolor infect. agent)

Mala f 3.0101 P56578 *Malassezia furfur* (Pityriasis versicolor infect. agent)  
Mala f 4.0101 Q9Y750 *Malassezia furfur* (Pityriasis versicolor infect. agent)  
Mala s 1.0101 Q01940 *Malassezia sympodialis*  
Mala s 10.0101 Q8TGH3 *Malassezia sympodialis*  
Mala s 11.0101 Q873M4 *Malassezia sympodialis*  
Mala s 12.0101 Q5GMY3 *Malassezia sympodialis*  
Mala s 13.0101 Q1RQI9 *Malassezia sympodialis*  
Mala s 5.0101 O93969 *Malassezia sympodialis*  
Mala s 6.0101 O93970 *Malassezia sympodialis*  
Mala s 7.0101 O93971 *Malassezia sympodialis*  
Mala s 8.0101 O93972 *Malassezia sympodialis*  
Mala s 9.0101 O93973 *Malassezia sympodialis*  
Mer a 1.0101 O49894 *Mercurialis annua* (Annual mercury)  
Met e 1.0101 Q25456 *Metapenaeus ensis* (Shrimp)  
Mor n 3.0101 P85894 *Morus nigra* (Mulberry)  
Mus a 1.0101 Q94JN3 *Musa acuminata* (Banana)  
Mus a 2.0101 Q8VXF1 *Musa acuminata* (Banana)  
Mus a 3.0101 P86333 *Musa acuminata* (Banana)  
Mus m 1.0101 P02762 *Mus musculus* (mouse)  
Mus m 1.0102 P11589 *Mus musculus* (mouse)  
Myr p 1.0101 Q07932 *Myrmecia pilosula* (Australian jumper ant)  
Myr p 2.0101 Q26464 *Myrmecia pilosula* (Australian jumper ant)  
Myr p 2.0102 Q26464 *Myrmecia pilosula* (Australian jumper ant)  
Myr p 3.0101 Q68Y22 *Myrmecia pilosula* (Australian jumper ant)  
Ole e 1.0101 P19963 *Olea europea* (Olive)  
Ole e 1.0105 P19963 *Olea europea* (Olive)  
Ole e 1.0106 P19963 *Olea europea* (Olive)  
Ole e 1.0107 P19963 *Olea europea* (Olive)  
Ole e 10.0101 Q84V39 *Olea europea* (Olive)  
Ole e 2.0101 O24169 *Olea europea* (Olive)  
Ole e 3.0101 O81092 *Olea europea* (Olive)  
Ole e 4.0101 P80741 *Olea europea* (Olive)  
Ole e 5.0101 P80740 *Olea europea* (Olive)  
Ole e 6.0101 O24172 *Olea europea* (Olive)  
Ole e 7.0101 P81430 *Olea europea* (Olive)  
Ole e 8.0101 Q9M7R0 *Olea europea* (Olive)  
Ole e 9.0101 Q94G86 *Olea europea* (Olive)  
Ory s 1.0101 Q40638 *Oryza sativa* (Rice)  
Ory s 12.0101 Q9FUD1 *Oryza sativa* (Rice)  
Pan s 1.0101 O61379 *Panulirus stimpsoni* (Spiny lobster)  
Par j 1.0101 P43217 *Parietaria judaica* (Pellitory-of-the-Wall)  
Par j 1.0102 O04404 *Parietaria judaica* (Pellitory-of-the-Wall)  
Par j 1.0103 Q1JTN5 *Parietaria judaica* (Pellitory-of-the-Wall)  
Par j 1.0201 Q40905 *Parietaria judaica* (Pellitory-of-the-Wall)  
Par j 2.0101 P55958 *Parietaria judaica* (Pellitory-of-the-Wall)  
Par j 2.0102 O04403 *Parietaria judaica* (Pellitory-of-the-Wall)  
Par j 3.0101 Q9XG85 *Parietaria judaica* (Pellitory-of-the-Wall)  
Par j 3.0102 Q9TOM8 *Parietaria judaica* (Pellitory-of-the-Wall)  
Par j 3.0201 L8BTD8 *Parietaria judaica* (Pellitory-of-the-Wall)  
Par j 4.0101 B5QST3 *Parietaria judaica* (Pellitory-of-the-Wall)  
Pas n 1.0101 B8PYF3 *Paspalum notatum* (Bahia grass)  
Pen b 26.0101 Q49KL9 *Penicillium brevicompactum*  
Pen c 13.0101 Q9URH1 *Penicillium citrinum*  
Pen c 19.0101 Q92260 *Penicillium citrinum*  
Pen c 22.0101 Q96X46 *Penicillium citrinum*  
Pen c 24.0101 Q69BZ7 *Penicillium citrinum*  
Pen c 3.0101 Q9Y8B8 *Penicillium citrinum*  
Pen c 30.0101 Q2V6Q5 *Penicillium citrinum*  
Pen ch 13.0101 Q9URR2 *Penicillium chrysogenum*  
Pen ch 18.0101 Q9P8G3 *Penicillium chrysogenum*  
Pen ch 20.0101 Q02352 *Penicillium chrysogenum*  
Pen ch 31.0101 Q2TL59 *Penicillium chrysogenum*  
Pen m 1.0101 A1KYZ2 *Penaeus monodon* (Black tiger shrimp)  
Pen m 2.0101 Q8I9P7 *Penaeus monodon* (Black tiger shrimp)  
Pen o 18.0101 Q9HF12 *Penicillium oxalicum*  
Per a 1.0101 Q9TZR6 *Periplaneta americana* (American cockroach)  
Per a 1.0102 O18535 *Periplaneta americana* (American cockroach)  
Per a 1.0103 O18530 *Periplaneta americana* (American cockroach)  
Per a 1.0104 O18528 *Periplaneta americana* (American cockroach)

Per a 1.0201 018527 *Periplaneta americana* (American cockroach)  
 Per a 3.0101 Q25641 *Periplaneta americana* (American cockroach)  
 Per a 3.0201 Q94643 *Periplaneta americana* (American cockroach)  
 Per a 3.0202 Q25640 *Periplaneta americana* (American cockroach)  
 Per a 3.0203 Q25639 *Periplaneta americana* (American cockroach)  
 Per a 6.0101 Q1M0Y3 *Periplaneta americana* (American cockroach)  
 Per a 7.0101 Q9UB83 *Periplaneta americana* (American cockroach)  
 Per a 7.0102 Q9UB83 *Periplaneta americana* (American cockroach)  
 Pers a 1.0101 P93680 *Persea americana* (Avocado)  
 Pha a 1.0101 Q41260 *Phalaris aquatica* (Canary grass)  
 Pha a 5.0101 P56164 *Phalaris aquatica* (Canary grass)  
 Pha v 3.0101 D3W146 *Phaseolus vulgaris* (Green bean, French bean)  
 Pha v 3.0201 D3W147 *Phaseolus vulgaris* (Green bean, French bean)  
 Phl p 1.0101 Q40967 *Phleum pratense* (Timothy)  
 Phl p 1.0102 P43213 *Phleum pratense* (Timothy)  
 Phl p 11.0101 Q8H6L7 *Phleum pratense* (Timothy)  
 Phl p 12.0101 P35079 *Phleum pratense* (Timothy)  
 Phl p 12.0102 Q24650 *Phleum pratense* (Timothy)  
 Phl p 12.0103 Q24282 *Phleum pratense* (Timothy)  
 Phl p 13.0101 Q9XG86 *Phleum pratense* (Timothy)  
 Phl p 2.0101 P43214 *Phleum pratense* (Timothy)  
 Phl p 4.0101 Q5ZQK5 *Phleum pratense* (Timothy)  
 Phl p 4.0201 Q5ZQK4 *Phleum pratense* (Timothy)  
 Phl p 5.0101 Q40960 *Phleum pratense* (Timothy)  
 Phl p 5.0102 Q40962 *Phleum pratense* (Timothy)  
 Phl p 5.0103 Q81341 *Phleum pratense* (Timothy)  
 Phl p 5.0104 P93467 *Phleum pratense* (Timothy)  
 Phl p 5.0105 Q65318 *Phleum pratense* (Timothy)  
 Phl p 5.0106 Q65319 *Phleum pratense* (Timothy)  
 Phl p 5.0107 Q65320 *Phleum pratense* (Timothy)  
 Phl p 5.0108 Q65321 *Phleum pratense* (Timothy)  
 Phl p 5.0109 Q84UT2 *Phleum pratense* (Timothy)  
 Phl p 5.0201 Q40963 *Phleum pratense* (Timothy)  
 Phl p 5.0202 P93466 *Phleum pratense* (Timothy)  
 Phl p 5.0203 Q81342 *Phleum pratense* (Timothy)  
 Phl p 5.0206 Q81343 *Phleum pratense* (Timothy)  
 Phl p 5.0207 Q81344 *Phleum pratense* (Timothy)  
 Phl p 6.0101 P43215 *Phleum pratense* (Timothy)  
 Phl p 6.0102 Q65868 *Phleum pratense* (Timothy)  
 Phl p 7.0101 Q82040 *Phleum pratense* (Timothy)  
 Pho d 2.0101 Q8L5D8 *Phoenix dactylifera* (Date palm)  
 Pis s 1.0101 Q702P1 *Pisum sativum* (Pea)  
 Pis s 1.0102 Q702P0 *Pisum sativum* (Pea)  
 Pis v 1.0101 B7P072 *Pistacia vera* (Pistachio)  
 Pis v 2.0101 B7P073 *Pistacia vera* (Pistachio)  
 Pis v 2.0201 B7P074 *Pistacia vera* (Pistachio)  
 Pis v 3.0101 B4X640 *Pistacia vera* (Pistachio)  
 Pis v 4.0101 B2BDZ8 *Pistacia vera* (Pistachio)  
 Pis v 5.0101 B7SLJ1 *Pistacia vera* (Pistachio)  
 Pla a 1.0101 Q8GT41 *Platanus acerifolia* (London plane tree)  
 Pla a 2.0101 Q6H9K0 *Platanus acerifolia* (London plane tree)  
 Pla l 1.0101 P82242 *Plantago lanceolata* (English plantain)  
 Pla l 1.0102 P82242 *Plantago lanceolata* (English plantain)  
 Pla l 1.0103 P82242 *Plantago lanceolata* (English plantain)  
 Pla or 1.0101 A9YUH4 *Platanus orientalis* (Oriental plane)  
 Pla or 2.0101 A9YUH5 *Platanus orientalis* (Oriental plane)  
 Pla or 3.0101 A9YUH6 *Platanus orientalis* (Oriental plane)  
 Plo i 1.0101 Q95PM9 *Plodia interpunctella* (Indianmeal moth)  
 Poa p 1.0101 Q9ZP03 *Poa pratensis* (Kentucky blue grass)  
 Poa p 5.0101 Q9FPR0 *Poa pratensis* (Kentucky blue grass)  
 Pol a 1.0101 Q9U6W0 *Polistes annularis* (Wasp)  
 Pol a 2.0101 Q9U6V9 *Polistes annularis* (Wasp)  
 Pol a 5.0101 Q05109 *Polistes annularis* (Wasp)  
 Pol d 1.0101 Q6Q252 *Polistes dominulus* (Mediterranean paper wasp)  
 Pol d 1.0102 Q6Q251 *Polistes dominulus* (Mediterranean paper wasp)  
 Pol d 1.0103 Q6Q250 *Polistes dominulus* (Mediterranean paper wasp)  
 Pol d 1.0104 Q6Q249 *Polistes dominulus* (Mediterranean paper wasp)  
 Pol d 4.0101 Q7Z269 *Polistes dominulus* (Mediterranean paper wasp)  
 Pol d 5.0101 Q68KJ8 *Polistes dominulus* (Mediterranean paper wasp)

Pol e 5.0101 Q68KJ9 *Polistes exclamans* (Wasp)  
 Pol f 5.0101 P35780 *Polistes fuscatus* (Wasp)  
 Pol g 1.0101 P83542 *Polistes gallicus* (Wasp)  
 Pol g 5.0101 P83377 *Polistes gallicus* (Wasp)  
 Pon l 4.0101 P05946 *Pontastacus leptodactylus* (Narrow-clawed crayfish)  
 Pru ar 1.0101 050001 *Prunus armeniaca* (Apricot)  
 Pru ar 3.0101 P81651 *Prunus armeniaca* (Apricot)  
 Pru av 1.0101 024248 *Prunus avium* (Sweet cherry)  
 Pru av 1.0201 Q6QHU3 *Prunus avium* (Sweet cherry)  
 Pru av 1.0202 Q6QHU2 *Prunus avium* (Sweet cherry)  
 Pru av 1.0203 Q6QHU1 *Prunus avium* (Sweet cherry)  
 Pru av 2.0101 P50694 *Prunus avium* (Sweet cherry)  
 Pru av 3.0101 Q9M5X8 *Prunus avium* (Sweet cherry)  
 Pru av 4.0101 Q9XF39 *Prunus avium* (Sweet cherry)  
 Pru d 3.0101 P82534 *Prunus domestica* (European plum)  
 Pru du 3.0101 C0LOI5 *Prunus dulcis* (Almond)  
 Pru du 4.0101 Q8GSL5 *Prunus dulcis* (Almond)  
 Pru du 4.0102 Q8GSL5 *Prunus dulcis* (Almond)  
 Pru du 5.0101 Q8H2B9 *Prunus dulcis* (Almond)  
 Pru p 1.0101 Q2I6V8 *Prunus persica* (Peach)  
 Pru p 3.0101 P81402 *Prunus persica* (Peach)  
 Pru p 4.0101 Q8GT40 *Prunus persica* (Peach)  
 Pru p 4.0201 Q8GT39 *Prunus persica* (Peach)  
 Pyr c 1.0101 065200 *Pyrus communis* (Pear)  
 Pyr c 3.0101 Q9M5X6 *Pyrus communis* (Pear)  
 Pyr c 4.0101 Q9XF38 *Pyrus communis* (Pear)  
 Pyr c 5.0101 081355 *Pyrus communis* (Pear)  
 Que a 1.0201 B6RQS1 *Quercus alba* (White oak)  
 Que a 1.0301 B6RQS2 *Quercus alba* (White oak)  
 Que a 1.0401 B6RQS3 *Quercus alba* (White oak)  
 Ran e 1.0101 Q8JIU2 *Rana esculenta* (edible frog)  
 Ran e 2.0101 Q8JIU1 *Rana esculenta* (edible frog)  
 Rat n 1.0101 P02761 *Rattus norvegicus* (Rat)  
 Rho m 1.0101 Q870B9 *Rhodotorula mucilaginosa* (Yeast)  
 Rho m 2.0101 Q32ZM1 *Rhodotorula mucilaginosa* (Yeast)  
 Ric c 1.0101 P01089 *Ricinus communis* (Castor bean)  
 Rub i 1.0101 Q0Z8U9 *Rubus idaeus* (Red raspberry)  
 Rub i 3.0101 Q0Z8V0 *Rubus idaeus* (Red raspberry)  
 Sal k 1.0101 P83181 *Salsola kali* (Russian thistle)  
 Sal k 1.0201 I6LD58 *Salsola kali* (Russian thistle)  
 Sal k 1.0301 Q17ST3 *Salsola kali* (Russian thistle)  
 Sal k 1.0302 Q17ST4 *Salsola kali* (Russian thistle)  
 Sal k 2.0101 Q8L5K9 *Salsola kali* (Russian thistle)  
 Sal k 3.0101 C1KEU0 *Salsola kali* (Russian thistle)  
 Sal k 4.0101 C6JWHO *Salsola kali* (Russian thistle)  
 Sal k 4.0201 E2D0Y9 *Salsola kali* (Russian thistle)  
 Sal k 5.0101 E2D0Z0 *Salsola kali* (Russian thistle)  
 Sal s 1.0101 Q91482 *Salmo salar* (Atlantic salmon)  
 Sar sa 1.0101 E3WFF7 *Sardinops sagax* (Pacific pilchard)  
 Seb m 1.0101 C6GKU4 *Sebastes marinus* (Ocean perch, redfish, snapper)  
 Seb m 1.0201 C6GKU5 *Sebastes marinus* (Ocean perch, redfish, snapper)  
 Sec c 38.0101 Q9S8H2 *Secale cereale* (Rye)  
 Sec c 20.0101 Q9S8B0 *Secale cereale* (Rye)  
 Sec c 20.0201 Q9S8A7 *Secale cereale* (Rye)  
 Ses i 1.0101 Q9AUD1 *Sesamum indicum* (Sesame)  
 Ses i 2.0101 Q9XHP1 *Sesamum indicum* (Sesame)  
 Ses i 3.0101 Q9AUD0 *Sesamum indicum* (Sesame)  
 Ses i 4.0101 Q9FUJ9 *Sesamum indicum* (Sesame)  
 Ses i 5.0101 Q9XHP2 *Sesamum indicum* (Sesame)  
 Ses i 6.0101 Q9XHP0 *Sesamum indicum* (Sesame)  
 Ses i 7.0101 Q9AUD2 *Sesamum indicum* (Sesame)  
 Sin a 1.0101 P15322 *Sinapis alba* (Yellow mustard)  
 Sin a 2.0101 Q2TLW0 *Sinapis alba* (Yellow mustard)  
 Sin a 3.0101 E6Y2L9 *Sinapis alba* (Yellow mustard)  
 Sin a 4.0101 E6Y2M0 *Sinapis alba* (Yellow mustard)  
 Sol g 4.0101 Q9NH75 *Solenopsis geminata* (Tropical fire ant)  
 Sol g 4.0201 Q9NH75 *Solenopsis geminata* (Tropical fire ant)  
 Sol i 1.0101 Q68KK0 *Solenopsis invicta* (Red imported fire ant)  
 Sol i 2.0101 P35775 *Solenopsis invicta* (Red imported fire ant)

Sol i 3.0101 P35778 *Solenopsis invicta* (Red imported fire ant)  
 Sol i 4.0101 P35777 *Solenopsis invicta* (Red imported fire ant)  
 Sol r 2.0101 P35776 *Solenopsis richteri* (Black fire ant)  
 Sol r 3.0101 P35779 *Solenopsis richteri* (Black fire ant)  
 Sola t 1.0101 P15476 *Solanum tuberosum* (Potato)  
 Sola t 2.0101 P16348 *Solanum tuberosum* (Potato)  
 Sola t 3.0101 024383 *Solanum tuberosum* (Potato)  
 Sola t 3.0102 P20347 *Solanum tuberosum* (Potato)  
 Sola t 4.0101 P30941 *Solanum tuberosum* (Potato)  
 Syr v 3.0101 P58171 *Syringa vulgaris* (Lilac)  
 Tha p 1.0101 Q7M4K8 *Thaumetopoea pityocampa* (Pine processionary moth)  
 Thu a 1.0101 C6GKU3 *Thunnus albacares* (Yellowfin tuna)  
 Tri a 12.0101 P49232 *Triticum aestivum* (Wheat)  
 Tri a 12.0102 P49233 *Triticum aestivum* (Wheat)  
 Tri a 12.0103 P49234 *Triticum aestivum* (Wheat)  
 Tri a 14.0201 D2T2K2 *Triticum aestivum* (Wheat)  
 Tri a 18.0101 P10968 *Triticum aestivum* (Wheat)  
 Tri a 25.0101 Q9LDX4 *Triticum aestivum* (Wheat)  
 Tri a 26.0101 P10388 *Triticum aestivum* (Wheat)  
 Tri a 27.0101 Q7Y1Z2 *Triticum aestivum* (Wheat)  
 Tri a 28.0101 Q4W0V7 *Triticum aestivum* (Wheat)  
 Tri a 29.0101 C7C4X0 *Triticum aestivum* (Wheat)  
 Tri a 29.0201 D2TGC2 *Triticum aestivum* (Wheat)  
 Tri a 30.0101 P17314 *Triticum aestivum* (Wheat)  
 Tri r 2.0101 Q9UW97 *Trichophyton rubrum*  
 Tri r 4.0101 Q9UW98 *Trichophyton rubrum*  
 Tri t 4.0101 P80514 *Trichophyton tonsurans*  
 Tria p 1.0101 Q9U6R6 *Triatoma protracta* (California kissing bug)  
 Tyr p 10.0101 Q6IUP9 *Tyrophagus putrescentiae* (Storage mite)  
 Tyr p 13.0101 Q66RP5 *Tyrophagus putrescentiae* (Storage mite)  
 Tyr p 2.0101 002380 *Tyrophagus putrescentiae* (Storage mite)  
 Tyr p 24.0101 D2DGW3 *Tyrophagus putrescentiae* (Storage mite)  
 Ves f 5.0101 P35783 *Vespula flavopilosa* (Yellow jacket)  
 Ves g 5.0101 P35784 *Vespula germanica* (Yellow jacket)  
 Ves m 1.0101 P51528 *Vespula maculifrons* (Yellow jacket)  
 Ves m 5.0101 P35760 *Vespula maculifrons* (Yellow jacket)  
 Ves p 5.0101 P35785 *Vespula pensylvanica* (Yellow jacket)  
 Ves s 5.0101 P35786 *Vespula squamosa* (Yellow jacket)  
 Ves v 1.0101 P49369 *Vespula vulgaris* (Yellow jacket)  
 Ves v 2.0101 P49370 *Vespula vulgaris* (Yellow jacket)  
 Ves v 2.0201 Q5D7H4 *Vespula vulgaris* (Yellow jacket)  
 Ves v 3.0101 B1A4F7 *Vespula vulgaris* (Yellow jacket)  
 Ves v 5.0101 Q05110 *Vespula vulgaris* (Yellow jacket)  
 Ves vi 5.0101 P35787 *Vespula vidua* (Wasp)  
 Vesp c 5.0101 P35781 *Vespa crabro* (European hornet)  
 Vesp c 5.0102 P35782 *Vespa crabro* (European hornet)  
 Vesp m 5.0101 P81657 *Vespa mandarinia* (Giant asian hornet)  
 Vig r 1.0101 Q2VU97 *Vigna radiata* (Mung bean)  
 Vit v 1.0101 P80274 *Vitis vinifera* (Grape)  
 Xip g 1.0101 B9W4C2 *Xiphias gladius* (Swordfish)  
 Zea m 1.0101 Q07154 *Zea mays* (Maize)  
 Zea m 12.0101 P35081 *Zea mays* (Maize)  
 Zea m 12.0102 P35082 *Zea mays* (Maize)  
 Zea m 12.0103 P35083 *Zea mays* (Maize)  
 Zea m 12.0104 022655 *Zea mays* (Maize)  
 Zea m 12.0105 Q9FR39 *Zea mays* (Maize)  
 Zea m 14.0101 P19656 *Zea mays* (Maize)  
 Zea m 14.0102 P19656 *Zea mays* (Maize)  
 Zea m 25.0101 Q4W1F7 *Zea mays* (Maize)  
 Ziz m 1.0101 Q2VST0 *Ziziphus mauritiana* (Chinese-date)  
 Cur l 4.0101 B3V0K8 *Curvularia lunata* (Synonym: *Cochliobolus lunatus*)  
 Pru du 6.0101 E3SH28 *Prunus dulcis* (Almond)  
 Pru du 6.0201 E3SH29 *Prunus dulcis* (Almond)  
 Pru p 2.0101 B6CQT7 *Prunus persica* (Peach)  
 Pru p 2.0201 B6CQT5 *Prunus persica* (Peach)  
 Pru p 2.0301 B6CQT3 *Prunus persica* (Peach)  
 Cas s 9.0101 Q9ZS24 *Castanea sativa* (Chestnut)  
 Ani s 10.0101 D2K835 *Anisakis simplex* (Nematode)  
 Tha p 2.0101 P86360 *Thaumetopoea pityocampa* (Pine processionary moth)

Glo m 5.0101 Q9NBA6 *Glossina morsitans* (Savannah Tsetse Fly)  
 Bomb m 1.0101 Q2F5T5 *Bombyx mori* (Silk moth)  
 Fag e 2.0101 Q2PSO7 *Fagopyrum esculentum* (Common buckwheat)  
 Len c 3.0101 A0AT29 *Lens culinaris* (Lentil)  
 Ole e 11.0101 D8VPP5 *Olea europea* (Olive)  
 Amb a 4.0101 D4IHC0 *Ambrosia artemisiifolia* (Short ragweed)  
 Pen m 4.0101 E7CGC4 *Penaeus monodon* (Black tiger shrimp)  
 Cla c 14.0101 G8Z4O7 *Cladosporium cladosporioides*  
 Pen ch 35.0101 G8Z4O8 *Penicillium chrysogenum*  
 Hev b 14.0101 E7BQV3 *Hevea brasiliensis* (Para rubber tree (latex))  
 Pen m 3.0101 E1A683 *Penaeus monodon* (Black tiger shrimp)  
 Pen m 6.0101 E7CGC5 *Penaeus monodon* (Black tiger shrimp)  
 Sola l 4.0101 O49881 *Solanum lycopersicum* (*Lycopersicon esculentum*) (Tomato)  
 Fag s 1.0101 B7TWE6 *Fagus sylvatica* (European beech)  
 Ost c 1.0101 E2GL17 *Ostrya carpinifolia* (European hophornbeam)  
 Lip b 1.0101 P86712 *Liposcelis bostrichophila* (Booklouse)  
 Tab y 2.0101 EOXKJ9 *Tabanus yao* (Horsefly)  
 Tab y 5.0101 EOXKJ8 *Tabanus yao* (Horsefly)  
 Ani s 11.0101 E9RFF3 *Anisakis simplex* (Nematode)  
 Ani s 12.0101 E9RFF6 *Anisakis simplex* (Nematode)  
 Gad m 1.0101 Q9OYLO *Gadus morhua* (Atlantic cod)  
 Gad m 1.0102 A5I873 *Gadus morhua* (Atlantic cod)  
 Gad m 1.0201 Q9OYK9 *Gadus morhua* (Atlantic cod)  
 Gad m 1.0202 A5I874 *Gadus morhua* (Atlantic cod)  
 Can f 6.0101 H2B3G5 *Canis familiaris* (dog)  
 Plo i 2.0101 E1XUQ3 *Plodia interpunctella* (Indianmeal moth)  
 Tyr p 3.0101 C6ZDB5 *Tyrophagus putrescentiae* (Storage mite)  
 Pan b 1.0101 E5BBS3 *Pandalus borealis* (Northern shrimp)  
 Tab y 1.0101 F1JZ10 *Tabanus yao* (Horsefly)  
 Tri a 15.0101 D2TGC3 *Triticum aestivum* (Wheat)  
 Tri a 21.0101 D2T2K3 *Triticum aestivum* (Wheat)  
 Tri a 31.0101 Q9FS79 *Triticum aestivum* (Wheat)  
 Tri a 32.0101 Q6W8Q2 *Triticum aestivum* (Wheat)  
 Tri a 33.0101 Q9ST57 *Triticum aestivum* (Wheat)  
 Tri a 34.0101 C7C4X1 *Triticum aestivum* (Wheat)  
 Tri a 35.0101 D2TE72 *Triticum aestivum* (Wheat)  
 Asp v 13.0101 D5LGB3 *Aspergillus versicolor*  
 Sta c 3.0101 C7E9W0 *Stachybotrys chartarum*  
 Cof a 1.0101 D7REL9 *Coffea arabica* (Arabian coffe)  
 Fag t 2.0101 E9NX73 *Fagopyrum tataricum* (Tartarian buckwheat)  
 Api g 6.0101 P868O9 *Apium graveolens* (Celery)  
 Man e 5.0101 M1E7Y0 *Manihot esculenta* (Cassava, manioc)  
 Onc m 1.0101 P86431 *Oncorhynchus mykiss* (Rainbow trout)  
 Onc m 1.0201 P86432 *Oncorhynchus mykiss* (Rainbow trout)  
 Api m 11.0101 B3GM11 *Apis mellifera* (Honey bee)  
 Api m 11.0201 Q4ZJX1 *Apis mellifera* (Honey bee)  
 Sal s 2.0101 B5DGQ7 *Salmo salar* (Atlantic salmon)  
 Tri a 37.0101 Q9TOP1 *Triticum aestivum* (Wheat)  
 Api m 12.0101 Q868N5 *Apis mellifera* (Honey bee)  
 Ves v 6.0101 G8IIT0 *Vespula vulgaris* (Yellow jacket)  
 Pen cr 26.0101 H2E5X2 *Penicillium crustosum*  
 Dau c 5.0101 H2DF86 *Daucus carota* (Carrot)  
 Fag e 3.0101 A5HIX6 *Fagopyrum esculentum* (Common buckwheat)  
 Vig r 2.0101 Q198W3 *Vigna radiata* (Mung bean)  
 Vig r 2.0201 B1NPN8 *Vigna radiata* (Mung bean)  
 Vig r 4.0101 Q43680 *Vigna radiata* (Mung bean)  
 Vig r 6.0101 Q9ZWP8 *Vigna radiata* (Mung bean)  
 Bra r 5.0101 P69197 *Brassica rapa* (Turnip)  
 Sch c 1.0101 D8Q9M3 *Schizophyllum commune*  
 Ore m 4.0101 K4PEK4 *Oreochromis mossambicus* (Mozambique tilapia)  
 Gad m 2.0101 B3AOL6 *Gadus morhua* (Atlantic cod)  
 Gad m 3.0101 P86980 *Gadus morhua* (Atlantic cod)  
 Sal s 3.0101 B5DGM7 *Salmo salar* (Atlantic salmon)  
 Thu a 2.0101 P86978 *Thunnus albacares* (Yellowfin tuna)  
 Thu a 3.0101 P86979 *Thunnus albacares* (Yellowfin tuna)  
 Bos d 9.0101 P02662 *Bos domesticus* (domestic cattle)  
 Bos d 10.0101 P02663 *Bos domesticus* (domestic cattle)  
 Bos d 11.0101 P02666 *Bos domesticus* (domestic cattle)  
 Bos d 12.0101 P02668 *Bos domesticus* (domestic cattle)

Pru p 7.0101 P86888 *Prunus persica* (Peach)  
 Bla g 3.0101 D0VNY7 *Blattella germanica* (German cockroach)  
 Gly m 7.0101 C6K8D1 *Glycine max* (Soybean)  
 Onc k 5.0101 D5MU14 *Oncorhynchus* (Chum salmon)  
 Cav p 4.0101 Q6WDN9 *Cavia porcellus* (guinea pig)  
 Ory c 3. Q9GK63 *Oryctolagus cuniculus* (rabbit)  
 Ory c 3. Q9GK67 *Oryctolagus cuniculus* (rabbit)  
 Por p 1.0101 M1H607 *Portunus pelagicus* (Blue swimmer crab)  
 Tri a 39.0101 J7QW61 *Triticum aestivum* (Wheat)  
 Der p 15.0101 Q4JK69 *Dermatophagoides pteronyssinus* (European house dust mite)  
 Der p 15.0102 Q4JK70 *Dermatophagoides pteronyssinus* (European house dust mite)  
 Der p 18.0101 Q4JK71 *Dermatophagoides pteronyssinus* (European house dust mite)  
 Cof a 2.0101 AGL34967 *Coffea arabica* (Arabian coffe)  
 Cof a 3.0101 AGL34968 *Coffea arabica* (Arabian coffe)  
 Lat c 1.0101 Q5IRB2 *Lates calcarifer* (Barramundi)  
 Lat c 1.0201 Q6ITU9 *Lates calcarifer* (Barramundi)

## E.1 Omitted allergens from allergen.org

A few of the entries were omitted, due to wrong accession codes, unpublished sequences or other errors:

Pen c 13.0101 Q9URH1 *Penicillium citrinum*  
 Api g 6.0101 P86809 *Apium graveolens* (Celery)  
 Gad m 2.0101 B3AOL6 *Gadus morhua* (Atlantic cod)  
 Gad m 3.0101 P86980 *Gadus morhua* (Atlantic cod)  
 Thu a 2.0101 P86978 *Thunnus albacares* (Yellowfin tuna)  
 Thu a 3.0101 P86979 *Thunnus albacares* (Yellowfin tuna)  
 Cof a 2.0101 AGL34967 *Coffea arabica* (Arabian coffe)  
 Cof a 3.0101 AGL34968 *Coffea arabica* (Arabian coffe)

## **F Results from the EFSA scientific opinion recommended allergen analysis of Protease from S10-34zEK4 using allergenonline database**

### **F.1 35% or larger identity over any 80 amino acid window**

(blank=No matches found) Count of significant hits described in text based on identity > 35%.

### **F.2 35% or larger identity over any 80 amino acid window (with scaling)**

(blank=No matches found) Count of significant hits described in text based on identity > 35%.

### **F.3 Identities calculated from Needleman-Wuncsh alignment**

Matches  $\geq$  10% are shown

## **G Results from the EFSA scientific opinion recommended allergen analysis of Protease from S10-34zEK4 using allergen.org database**

### **G.1 35% or larger identity over any 80 amino acid window**

(blank=No matches found) Count of significant hits described in text based on identity > 35%.

### **G.2 35% or larger identity over any 80 amino acid window (with scaling)**

(blank=No matches found) Count of significant hits described in text based on scaled identity > 35%.

### **G.3 Identities calculated from Needleman-Wuncsh alignment**

Matches  $\geq$  10% are shown

–

Esben Peter Friis <epf@novozymes.com>

May 15, 2014

**Toxicology**

Date: 07 March 2013  
File: 2013-03986-01  
Ref.: LiNi/PBjP/TrGQ

## **SUMMARY OF TOXICITY DATA**

**Serine endopeptidase, batch PPA 26797**  
***Bacillus licheniformis***



*Issued by:*  
**Toxicology**  
**Novozymes A/S**  
**Krogshøjvej 36**  
**2880 Bagsvaerd**  
**Denmark**

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## SUMMARY

The below series of toxicological studies were undertaken to evaluate the safety of Serine endopeptidase (Chymotrypsin/10R Protease), represented by batch PPA 26797.

All studies were carried out in accordance with current the OECD guidelines and in compliance with the OECD principles of Good Laboratory Practice (GLP). The studies were carried out at Covance, England and at Novozymes A/S, Denmark, during the period August 2007 to October 2007.

The main conclusions of the safety studies can be summarized as follows:

- Serine endopeptidase, batch PPA 26797 gave no indication of mutagenic activity when tested in the bacterial reverse mutation assay in the presence or absence of S9 mix.
- Serine endopeptidase, batch PPA 26797 did not show any clastogenic activity, neither in the presence or absence of S-9 mix, when tested in the *in vitro* chromosome aberration test.
- In a 13-weeks oral toxicity study in rats Serine endopeptidase, batch PPA 26797 was well tolerated and did not cause any toxicologically significant changes at any dose level.

## 2. TEST SUBSTANCE

Serine endopeptidase, batch PPA 26797 is a liquid enzyme concentrate produced in *Bacillus licheniformis*. The principal enzyme activity is serine endopeptidase, PROT/g (EC 3.4.21).

### 2.1 Production microorganism

This protease is produced by a strain of *Bacillus licheniformis* containing the chromosomally integrated gene encoding a proteolytic enzyme originating from a strain of the actinomycete *Nocardioopsis prasina*.

This genetically modified production strain meets the criteria for a safe production microorganism. It is constructed by common transformation procedures using well-known plasmid vectors with strictly defined and well-characterized DNA sequences that are not known to encode or express any harmful or toxic substances.

*Bacillus licheniformis* has long history of safe use. This species has been used for decades in the production of enzymes, and for more than a decade as recombinant production organism. It is generally regarded as non-pathogenic and non-toxicogenic. Further investigations, carried out according to EC recommendations have revealed that the recipient strain applied does not produce any known *Bacillus* toxins. Therefore, according to EFSA classification, the production strain can be considered QPS.

The enzyme product does not contain the production strain or recombinant DNA, and their absence is part of the complete specification of the product.

## 2.2 Characterisation

The characterization of the batch PPA 26797 used for the conduct of the toxicological studies is presented in Table 1.

**Table 1.**

Characterization of Serine endopeptidase, batch PPA 26797

Batch number	PPA 26797
Activity, PROT/g	54600
Water (KF) (% w/w)	88.1
Ash (600°C) (% w/w)	2.4
Total Organic Solids (TOS <sup>1</sup> )	9.5
Specific gravity (g/mL)	1.053

<sup>1</sup> % TOS is calculated as 100% - % water - % ash - % diluents.

## 3. MUTAGENICITY

### 3.1 Bacterial Reverse Mutation Assay

Serine endopeptidase, batch PPA 26797 was examined for mutagenic activity using *Salmonella typhimurium* strain TA1535, TA100, TA1537, TA98 and *Escherichia coli* WP2uvrA.

The study was conducted using the treat and plate assay, in the presence and absence of metabolic activation (a liver preparation from male rats, pre-treated with Aroclor1254, and the co-factors required for mixed function oxidase activity (S9 mix)).

All bacterial strains were tested at concentrations of the test article ranging from 156 to 5000 µg per plate. All results were confirmed by conducting two complete and independent experiments.

The sensitivity of the individual bacterial strains was confirmed by incorporation of diagnostic mutagens. In all test series these positive control substances induced significant and satisfactory responses in the appropriate strains under similar test conditions as the test article.

No significant, dose-related and reproducible increases in the number of revertant colonies compared to the solvent control were obtained with any of the bacterial strains exposed to the test substance, neither in the presence or absence of S9 mix.

It was concluded that the results of the experiment give no indication of mutagenic activity of Serine endopeptidase, batch PPA 26797 in the presence or absence of S9 mix, when tested under the conditions employed in the study.

### 3.2 *In vitro* Chromosome Aberration Assay

In order to assess the clastogenic activity of Serine endopeptidase, batch PPA 26797, an *in vitro* chromosome aberration assay was performed.

Two independent experiments were conducted with and without the inclusion of metabolic activation (S-9 mix). In the first experiment, cells were exposed for 3 hours

followed by a 17 hours recovery period. In the second experiment, a continued exposure time of 20 hours (with no recovery period) was included without S-9 mix. With S-9 mix, exposure was limited to 3 hours and 17 hours recovery as in experiment 1.

The dose levels for chromosome analysis were selected by evaluating the effect of the test substance on mitotic index. The highest concentration for analysis was 5000 µg/mL and 4000 µg/mL (expressed in terms of the test substance as supplied) for the short and continued treatment periods, respectively. Chromosome aberrations were scored by examination of 100 metaphases per culture, and the frequencies of cells with one or more aberrations were calculated both including and excluding gap-type aberrations.

No increases in the frequency of cells with chromosomal or numerical aberrations, considered to be of any biological importance, were observed.

It was concluded that Serine endopeptidase, batch PPA 26797 did not induce chromosome aberrations in cultured human blood lymphocytes when tested up to to 5000 µg/mL in the presence and absence of S-9 mix.

## **4. GENERAL TOXICITY**

### **4.1 A 13-Week Oral (Gavage) Toxicity Study in Rats**

The objective of this study was to assess the systemic toxic potential of Serine endopeptidase, PPA 26797 when administered orally by gavage to rats for 13 weeks.

The study was carried out in accordance with the OECD guideline 408 (adopted on September 1998). It was conducted in accordance with Good Laboratory Practice.

Three groups each of ten male and ten female rats received Serine endopeptidase PPA 26797 at dose levels of 50, 165.1 and 500.1 mg Total Organic Solids (TOS) per kg body weight (bw) per day (equivalent to 28747, 90090 and 287469 PROT /kg bw/day) for thirteen weeks. The dose volume was 5 ml/kg bw/day. A similarly constituted group received the vehicle (tap water) at the same volume dosage and served as the negative control.

Clinical signs were recorded daily. Body weights and food consumption were recorded once weekly. Water consumption was recorded twice weekly. Towards the end of the study, all animals were subjected to open-field-testing and stimuli-induced tests. Ophthalmoscopy was performed on all animals before start of treatment, and on the animals of groups 1 and 4 at the end of treatment. Before termination of treatment, blood samples were taken for hematology and clinical chemistry. The animals were killed and subjected to a macroscopic necropsy. Specified organs and tissues were weighed, fixed and prepared for histopathological examination.

No treatment related effects were seen at the clinical examinations, on the body weight, body weight gain, on the food consumption, water consumption, on the clinical pathological parameters, at the ophthalmoscopic examination or on the organ weights.

At necropsy, no treatment related macroscopic changes were found. All microscopic findings reported were considered to be within the background incidence of findings

reported in this age and strain of laboratory maintained rats and as such to be of no toxicological significance.

In conclusion, thirteen weeks of oral administration (by gavage) Serine endopeptidase PPA 26797 at dose levels of up to 500.1 mg Total Organic Solids (TOS) per kg body weight (bw) per day (equivalent to 287469 PROT/kg bw/day) resulted in no treatment-related effects. In this study the no observed effect level (NOEL) in rats treated orally by gavage for 13 weeks was considered to be the highest dose level administered, equivalent to 5 mL 100% batch/kg bw/day or 500.1 mg Total Organic Solids (TOS) per kg body weight (bw) or 287469 PROT /kg bw/day.

## 7. REFERENCES

### 7.1 Studies

Novozymes A/S: Study No.: 20078045. Serine endopeptidase PPA 26797: Test for Mutagenic Activity with Strains of *Salmonella typhimurium* and *Escherichia coli*. (August 2007) Luna file: 2007-38794

Covance Laboratories: Study no. 1974/62. Novozymes Reference no. 20076030. Serine endopeptidase PPA 26797: Induction of chromosome aberrations in cultured human peripheral blood lymphocytes (September 2007) Luna file: 2007-38802

Novozymes A/S: Study No.: 20076029. Serine endopeptidase PPA 26797: A 13-Week Oral (Gavage) Toxicity Study in Rats. (October 2007) Luna file: 2007-42583.

### 7.2 Guidelines

OECD principles of Good Laboratory Practice (GLP) (as revised in 1997), ENV/MC/CHEM(98)17. OECD, Paris.

OECD, Guidelines for testing of Chemicals. Section 4: Health effects. Organisation for Economic Co-operation and Development, Paris.

**Safety & Toxicology**

Date: 07.July.2009  
Proj: Dev 00632  
File: 2007-38794-02  
Ref.: UF/PBJP

## REPORT

### Amended

**Serine Endopeptidase, PPA 26797:  
Test for Mutagenic Activity with Strains of  
*Salmonella typhimurium* and *Escherichia coli*.**

**Study No. 20078045**



*Issued by :*  
**Novozymes A/S  
Krogshøjvej 36  
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## GLP - COMPLIANCE STATEMENT

**REPORT:** Serine Endopeptidase, PPA 26797: Test for Mutagenic Activity with Strains of *Salmonella typhimurium* and *Escherichia coli* .

**STUDY No.:** 20078045

A sample of Serine Endopeptidase Batch Number: PPA 26797 was received from Recovery Pilot Plant, Novozymes A/S.

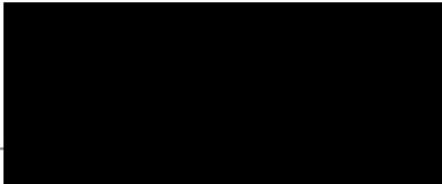
This study was conducted at the department of Safety & Toxicology, Novozymes A/S in compliance with the following current Good Laboratory Practice Regulations:

OECD, ENV/MC/CHEM(98)17, 1998

The study was first reported on 20070919. This amended final report was issued on 20090708 for the following reasons:

For confidentiality reasons the internal Novozymes name, used in the development phase and toxicological studies of the enzyme, has been taken out of the present study report and has been replaced by the generic enzyme name – Serine Endopeptidase, followed by an unambiguous identification number of the test batch (PPA 26797). The sample identification number of the test batch is exactly the same as used in the original report. These changes affect neither the original data nor the original conclusions. No other substitutions have been made in the amended report.

Date: 8 July 2009

  
Study Director

# QUALITY ASSURANCE STATEMENT

Report: Serine Endopeptidase, PPA 26797  
Test for Mutagenic Activity with Strains of Salmonella  
typhimurium and Escherichia coli.

STUDY NUMBER 20078045

The conduct of this study has been subject to appropriate inspections and the report has been reviewed according to the relevant Standard Operation Procedures of Novozymes A/S Quality Assurance.

Inspection/Audit	Dates of inspection	Dates of Audit Report signed by Study Director	Dates of Audit Report signed by Study Management
Protocol	7 JUN 2007	17 SEP 2007	18 SEP 2007
Inoculation of test culture	28 JUN 2007	17 SEP 2007	18 SEP 2007
Report	13 SEP 2007	17 SEP 2007	18 SEP 2007
Report - Amended	7 JUL 2009	7 JUL 2009	7 JUL 2009

8 July 2009  
Date



Quality Assurance

## 1. GENERAL INFORMATION

**STUDY** Serine Endopeptidase, PPA 26797: Test for Mutagenic Activity with Strains of *Salmonella typhimurium* and *Escherichia coli*.  
Study No. 20078045

**STUDY DIRECTOR** [REDACTED]  
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Novozymes A/S  
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**DATES OF STUDY** Study initiation date: 31.May.2007  
Experimental start date: 07.June.2007  
Experimental termination: 31.August.2007

### PERSONNEL INVOLVED IN THE STUDY

**TECHNICIANS** [REDACTED] Safety & Toxicology  
[REDACTED] (PScK) – Safety & Toxicology  
[REDACTED] Safety & Toxicology

### DATE OF FINAL REPORT

Date: 8. July 2009

[REDACTED]  
Safety & Toxicology

## 2. SUMMARY

Serine Endopeptidase (Batch Number: PPA 26797) was examined for mutagenic activity in the bacterial reverse mutation assay using *Salmonella typhimurium* strain TA1535, TA100, TA1537, TA98 and *Escherichia coli* WP2uvrApKM101.

The study was conducted, using the treat and plate assay, in the presence and absence of metabolic activation - a liver preparation from male rats, pre-treated with Aroclor 1254, and the co-factors required for mixed function oxidase activity (S9 mix). All bacterial strains were tested at concentration of the test article ranging from 156 to 5000 µg per plate.

All results were confirmed by conducting two complete and independent experiments.

We consider a test substance as positive in this treat and plate assay when it has induced at least a doubling in the mean number of revertants per plate compared to the appropriate solvent control in one or more of the strains, in the presence or absence of S9, if this response is dose related and reproducible. If a dose related numerical increase below a doubling but at least 50% higher than the solvent control is observed then the result is considered as equivocal and need further clarification.

No treatments of any of the *Salmonella* and *E.coli* strains with Serine Endopeptidase (Batch PPA 26797) either in the presence or absence of S-9 mix, resulted in any increases in revertant numbers that meets these criteria for a positive response.

It was concluded, that the results of the experiments, described in this report, give no indication of mutagenic activity of Serine Endopeptidase (Batch Number: PPA 26797) in the presence or absence of metabolic activation, when tested under the conditions employed in this study.

### 3. INTRODUCTION

Bacterial reverse mutation assays have been recognised and used for more than two decades as a rapid, sensitive and reliable method of evaluating the mutagenic potential of chemicals. Bacterial systems offer several advantages to other test systems. They can be grown in large numbers in a short time, enabling the detection of very rare mutational events. Further, extensive knowledge of bacterial genetics has allowed the construction of special strains, which are more sensitive than are wild-type strains to a variety of agents.

The reversion of bacteria from growth-dependence on a particular amino acid to growth in the absence of that amino acid is the most widely used marker in reverse-mutation assays. The genetic target is small, specific and selective, and the phenotypic effect of the reverse mutation is easily detected.

A wide range of strains within the species *Salmonella typhimurium* (Ames strains) and *Escherichia coli* have been constructed in order to make the test system more sensitive and selective to different classes of chemical mutagens.

By incorporation of the post-mitochondrial supernatant (S9) from the livers of rats pre-treated with an enzyme inducer Aroclor 1254, the metabolising systems present in mammalian cells are mimicked to facilitate the detection of a wide range of pro-mutagens.

This report describes experiments performed to assess the activity of Serine Endopeptidase (Batch Number: PPA 26797) in amino acid dependent strains of *Salmonella typhimurium* and *Escherichia coli* capable of detecting both induced frame-shift (TA1537 and TA98) and base-pair substitution mutations (TA1535, TA100, and WP2uvrApKM101).

Serine Endopeptidase (Batch No. PPA 26797) is a microbial enzyme preparation derived from submerged pure culture fermentation of a non-pathogenic and non-toxigenic strain. It contains a variety of unspent medium residues, including low concentrations of free amino acids like histidine and tryptophan.

This complexity poses several problems during mutagenicity testing in vitro. In the Ames test it composes a rich growth medium to the test bacteria, resulting in completely different and poorly defined environments of exposed cultures compared to control cultures. The main problem is the content of utilizable histidine and tryptophan in the test material, since the principle of the Ames test is the histidine auxotrophy of the *Salmonella* tester strains and tryptophan auxotrophy of the *E.coli* strains.

As a result, the density of the bacterial background lawn increase with increasing doses ("feeding effect") followed by dose related increases in the number of spontaneous revertant. These increases are obviously artificial.

To overcome this problem all the bacterial strains applied in the present study were treated with Serine Endopeptidase in liquid culture ("treat and plate assay").

The study was conducted in accordance with OECD Guideline for testing of chemicals, No. 471: Bacterial Reverse Mutation Assay" (July 1997 concerning the general specifications of the test. However the exposure of test bacteria in liquid culture ("treat and plate") is not specifically described in any guidelines.

### 4. MATERIALS

#### 4.1. Test substance

Serine Endopeptidase (Batch Number: PPA 26797) was received from Recovery Pilot Plant on the 18.Apr.2007, and immediately stored in a freezer. The substance was a brown liquid with a declared content of 11.9 % (w/w) dry matter.

A standard solution of 5% (w/v) dry matter was prepared in deionised water and sterilised by filtration.

Samples were sterilised by filtration and the sterility was confirmed by plate counting. Solution was stored at 4°C and used as test substance.

#### 4.2. Positive control substances

Chemical	Source	Lot.No.
2-Nitrofluorene (2-NF)	Aldrich-Chemie	S 08447-186
9-Aminoacridine (9-AA)	SIGMA Chemical Company	106F-06682
N-Ethyl-N'-Nitro-N-Nitrosoguanidine (ENNG)	Aldrich-Chemie	08228 ES
2-Aminoanthracene (2-AA)	SIGMA Chemical Company	S 11804-492
N-Methyl-N'-Nitrosoguanidine – MNNG	Aldrich-Chemie	15427 LO

All positive control substances were dissolved in dimethyl sulphoxide (spectrophotometric grade) obtained from Merck, Darmstadt, Germany.

#### 4.3. Liver homogenate - S9

A commercial preparation of S9 from Aroclor 1254 induced Sprague Dawley rats was obtained from MP Biomedicals, LLC. 29525 Fountain Parkway Solon, Ohio 11439. Specifications of the preparation, the enzymatic properties and metabolic activation from the supplier are archived as raw data.

The tubes with S9 were received frozen in dry ice and were immediately stored in a  $-80^{\circ}\text{C}$  ultra low freezer at Safety & Toxicology, Novozymes.

#### 4.4. Plates

As selective substrate for reverted bacteria Vogel-Bonner medium E agar plates with 2% glucose were prepared in-house as described in Appendix 3.

All plates were stored at  $4^{\circ}\text{C}$  in closed plastic bags and examined for contamination and dryness before use.

#### 4.5. Bacteria

##### *Salmonella typhimurium*

Four strains of *Salmonella typhimurium* were used:

*S. typhimurium* TA1535

*S. typhimurium* TA100

*S. typhimurium* TA1537

*S. typhimurium* TA98

All these strains contain mutations in the histidine operon, thereby imposing a requirement for histidine in the growth medium. They all contain GC base-pairs at the site of the histidine mutation, and are therefore selective for agents which react predominantly with these bases. Three mutations in the histidine operon are involved:

his G 46 (TA1535 and TA100) is a missense mutation which is reverted to prototrophy by a variety of mutagens that cause base-pair substitutions.

his C 3076 (TA1537) contains a frame-shift which appears to have added a GC base-pair. This mutation is reverted for example by 9-Aminoacridine and epoxides of polycyclic hydrocarbon.

his D 3052 (TA98) also contains a frame-shift mutation with a sequence of repeated GC, which is reverted with the deletion of 2 of these base-pairs. It is readily reverted by aromatic amines and derivatives.

All 4 strains contain the deep rough (*rfa*) mutation, which deletes the polysaccharide side chain of the polysaccharide coat of the bacterial cell surface. This deletion increases cell permeability to more hydrophobic substances and, furthermore, greatly decreases the pathogenicity of these organisms.

The *uvrB* deletion renders the strains incapable of excision repair, making them more sensitive both to the mutagenic and lethal effects of a wide variety of mutagens (e.g. poly-aromatic hydrocarbons), since the strains can not excise DNA adducts. These 2 deletions include the nitrate reductase (*chl*) and biotin (*bio*) genes also.

Strain TA98 and TA100 are derived from strain TA1538 and TA1535 respectively by the addition of a plasmid, pKM101, which confers resistance to ampicillin. This plasmid also carries a gene (*muc*<sup>+</sup>), which in some strains (*recA*<sup>+</sup>/*lexA*<sup>+</sup>) have proven to participate in "SOS" DNA-repair. This repair pathway is induced by DNA damage and confers resistance to the lethal effects of many mutagens at the expense of increased mutability. Bacteria carrying pKM101 have therefore a higher spontaneous mutation rate.

### *Escherichia coli*

One strain was used:  
*Escherichia coli* WP2uvrApKM101

This strain contain an ochre mutation in the *trpE* locus and can be mutated to tryptophan independence either by a base-pair reversion of an A-T base-pair in the *trpE* locus, or more likely, by a base-pair substitution within a number of transfer RNA loci elsewhere in the chromosome. The latter causes the original defect to be suppressed (ochre suppression) and involves only base-pair substitution transitions at G-C base-pairs. Like the *uvrB* mutation in the *Salmonella* strains, the *uvrA* mutation causes the bacteria to be deficient in the excision of bulky lesions from the DNA, so, it is more readily mutated by certain agents (ultraviolet radiation, polycyclic hydrocarbons). As indicated by the designation WP2uvrApKM101 this strain also contains the plasmid pKM101 as described for *Salmonella* TA98 and TA100.

#### 4.6. Bacterial cultures

The test strains of *Salmonella typhimurium* LT2 were obtained from Prof. B.N. Ames, Biochemistry Department, University of California, Berkeley, CA 94720, U.S.A.

*Escherichia coli* WP2uvrApKM101 was received from Covance Laboratories, Harrogate, UK and originally obtained from The National Collections of Industrial and Marine Bacteria Ltd., Aberdeen, Scotland.

New batches of culture stocks frozen in 8% dimethyl sulphoxide are prepared at intervals from a central stock held in liquid nitrogen. They are regularly checked for appropriate amino acid requirement, spontaneous reversion rate, genetic characters and response to diagnostic mutagens.

Samples of each strain were grown up overnight in Nutrient broth in a 37 ± 1°C water bath with shaking. Fresh cultures were prepared before each test.

#### 4.7. S9 mix

Composition of a 10% V/V S9 mix (final concentrations):

Co-factors:

-phosphate buffer (0.2M, pH 7.4) ..... 100 mM  
-salts (1.65M KCl, 0.4 M MgCl)..... 33 and 8 mM  
-glucose-6-phosphate, mono-Na salt (0.2M) ..... 5 mM  
-NADP, di-Na salt (0.1M) ..... 4 mM  
S9 preparation ..... 10% V/V

A freshly prepared solution of the co-factors was filter-sterilised by passage through a 0.2 µm membrane filter and mixed 9:1 (v/v) with freshly thawed still cold S9 preparation.

This S9 mix was prepared freshly each day, and immediately used. Unused reagent was discarded.

#### 4.8. Test material

Serial dilutions of the sterile standard solution (4.1.) were prepared in sterile deionised water corresponding to the final dose levels:

5000 µg - 2500 µg - 1250 µg - 625 µg - 313 µg - 156 µg substance per ml.

The dilutions were prepared freshly each day just before use.

This range of doses was applied in experiments with respectively without S9.

#### 4.9. Top agar

0.6 % soft agar was sterilised by autoclaving.

Bottles with 100 ml melted soft agar were kept at about 55°C and added 0.5 mM 10 ml L-histidine/biotin solution for strains of *Salmonella* or 10 ml 0.5 mM tryptophan solution for *Escherichia coli*. This molten agar was divided into 2 ml aliquots in sterile glass tubes and placed in a "Digital heatblock" (VWR) at 45 ± 1°C.

### 5. METHODS

#### 5.1. Treat and plate assay

For each assay sterile tubes were added:

- 4 ml Nutrient broth
- 4 ml S9 mix or 0.2M phosphate buffer (pH 7.4)
- 1 ml bacterial culture
- 1 ml test substance solution (6 doses) or diagnostic mutagen solution (positive control) or sterile deionised water (solvent control).

These incubation mixtures were incubated with shaking at 37 ± 1°C for 3 hours.

After incubation all bacterial suspensions were washed 2 times by centrifugation for 10 minutes at 2500 rpm. After the first washing the bacterial pellets were resuspended in 5 ml phosphate buffer (pH 7.4, 0.2M) and finally they were re-suspended in 1 ml phosphate buffer.

Tubes with top agar were added 0.1 ml of all washed bacterial suspensions.

#### 5.3. Selective incubation

For each dose of the test substance and the standard mutagens 3 similar tubes with top agar were prepared and 5 tubes were prepared for the solvent control.

These tubes were poured on to minimal glucose agar plates. When the soft agar set, the plates were inverted and incubated at 37 ± 1°C for about 64 hours. After incubation the numbers of revertant colonies were counted automatically ("Cardinal" - Perceptive Instruments). Plates with less than about 20 colonies were counted manually.

#### 5.4. Viable cell count

0.1 ml aliquots of a 10<sup>-6</sup> dilution of each bacterial suspension were poured on to minimal glucose agar plates (added the required amino acids in excess) in duplicates.

#### 5.5. Controls

The following controls were run with each experiment:

##### Genotype checking:

- Sensitivity for crystal violet (*rfa*-character), (except *E.coli*)
- Sensitivity for Mitomycin C (*uvrB*).
- Resistance to ampicillin

0.1 ml bacterial culture was spread on to complete agar medium. To the surface of the dried plate was added a disc of ampicillin/(Rosco Neo-Sensitabs) and two 6 mm φ sterile filter discs, one with 10µl 0.1% crystal violet and the other with 10µl 0.01% Mitomycin C. The plate was incubated for 64-72 hours at 37 ± 1°C.

**Sterility of S9 mix:**

0.1 ml S9 mix was plated on to complete medium and incubated for 64-72 hours at 37 ± 1°C.

Diagnostic mutagens were used for each strain with and without S9 mix, as follows:

Mutagen	S9	Strain	µg/mL
MNNG	-	TA 1535	1.0
MNNG	-	TA 100	1.0
2-NF	-	TA 98	20.0
9-AA	-	TA 1537	2.0
MNNG	-	WP2uvrApKM101	7.5
2-AA	+	TA 98	5.0
2-AA	+	TA 1537	5.0
2-AA	+	TA 1535	5.0
2-AA	+	TA 100	5.0
2-AA	+	WP2uvrApKM101	20.0

## 6. RESULTS AND DISCUSSION

### 6.1 Genetic characters

All *Salmonella* strains used in these experiments were sensitive to crystal violet and Mitomycin C. TA98, TA100 and the *E.coli* WP2uvrApKM101 strain were all resistant to ampicillin. The *E.coli* strain was sensitive to Mitomycin C. These results are as expected.

### 6.2 Negative control levels

The solvent control values presented in this report are within the range of our historical data for the treat and plate assay. In the second experiment with TA1535 with S-9 the solvent control just exceeded the range experienced in our laboratory in the near past (Appendix 1). However it is well within the corresponding level without S-9 and the level for the standard plate incorporation method as reported in the literature and experienced in our laboratory.

### 6.3 Diagnostic mutagens

In general the positive control chemicals induced significant increases in revertant colony numbers thereby confirming the sensitivity of the test system. In the second experiment with *S.typhimurium* TA 1535 without S-9, 1 µg/ml MNNG induced a significant positive response, about fifteen times the solvent control level, but in the low end of our historical data (Appendix 2). On the contrary in the first experiment the corresponding data exceeded the normal range experienced in our laboratory.

### 6.4 Serine Endopeptidase (Batch Number: PPA 26797 )

The results are represented in Table 1-10.

Serine Endopeptidase is a fluid enzyme preparation. It contains an abundance of various nutrients, and composes a rich growth medium to the test bacteria. This means, that comparison of viable counts between exposed cultures and control culture in a "treat and plate" assay reflects growth stimulation/inhibition as well as cell killing. Variation in the viable counts may cause some variation in the number of spontaneous revertant colonies.

It is our experience, that in a treat and plate assay, where bacteria are exposed to different doses of such a test substance in separate liquid cultures for a certain time, the spontaneous revertant levels fluctuate more than in the direct "plate incorporation assay."

Based on the viable counts of the treated culture no distinct toxicity was observed in the present study. Reduced viabilities were observed mainly in tests with S-9 incorporation and with the two base-pair substitution strain TA100 and TA1535 of *Salmonella* but only at the highest dose levels. As a consequence the number of connected revertant colonies was relatively low.

We consider a test substance as positive in this treat and plate assay when it has induced at least a doubling in the mean number of revertants per plate compared to the appropriate solvent control in one or more of the strains, in the presence or absence of S9, if this response is dose related (at least 3 doses) and reproducible. If a numerical increase below a doubling is observed the result is considered as equivocal and need further clarification if the increase is dose related and reproducible and it is not accompanied by significant increases in the viable bacterial count.

No treatments of any of the *Salmonella* and *E.coli* strains with Serine Endopeptidase resulted in any increases in revertant numbers that meets these criteria for a positive or equivocal response.

## 7. CONCLUSION

The results of the bacterial mutagenicity tests described in this report give no indication of the presence of mutagenic components in this preparation of Serine Endopeptidase (Batch No. PPA 26797), when tested under the conditions employed in this study.

**Table 1.** Number of revertant colonies per plate obtained with *Salmonella typhimurium* following exposure to Serine Endopeptidase (Batch No. PPA 26797) in the absence of metabolic activation in the treat and plate assay.

**1. experiment. Without S9 Mix**

Test Substance Concentration µg per mL	Number of revertants (number of colonies/plate) Base-pair substitution type							
	TA100				TA1535			
	Revertants <sup>1)</sup>		Viable cells <sup>2)</sup>		Revertants <sup>1)</sup>		Viable cells <sup>2)</sup>	
	Single plates	Mean	Single plates	Mean	Single plates	Mean	Single plates	Mean
5000	90	109	72	75	7	7	126	116
	114		77		8		105	
	124				6			
2500	115	110	65	67	9	6	114	104
	107		69		6		93	
	107				4			
1250	159	157	124	128	11	10	123	111
	174		132		9		99	
	137				9			
625	144	148	205	197	2	8	202	116
	153		188		13		229	
	147				9			
313	141	143	119	136	13	9	153	145
	163		153		7		137	
	126				8			
156	167	162	119	125	11	12	152	168
	140		130		12		184	
	179				14			
Solvent control	140	157	86	86	12	8	148	145
	135				8			
	156				3			
	183				5			
	169				10			
MNNG 1.0 µg	4797	4910	82	81	6497	6356	177	168
	4984		79		6226		159	
	4949				6345			

Abbreviations:

MNNG: N-Methyl-N'-nitro-N-nitrosoguanidine

**Table 2.** Number of revertant colonies per plate obtained with *Salmonella typhimurium* following exposure to Serine Endopeptidase (Batch No. PPA 26797) in the presence of metabolic activation in the treat and plate assay.

**1. experiment. With S9 Mix**

Test Substance Concentration µg per mL	Number of revertants (number of colonies/plate) Base-pair substitution type							
	TA100				TA1535			
	Revertants <sup>1)</sup>		Viable cells <sup>2)</sup>		Revertants <sup>1)</sup>		Viable cells <sup>2)</sup>	
	Single plates	Mean	Single plates	Mean	Single plates	Mean	Single plates	Mean
5000	132	155	186	182	6	8	204	214
	161		178		9		223	
	173				9			
2500	136	128	125	136	13	11	213	221
	129		147		14		229	
	120				6			
1250	112	117	140	150	9	7	213	230
	123		159		6		247	
	117				5			
625	156	153	134	145	5	8	228	246
	152		156		10		264	
	152				8			
313	124	118	152	144	12	11	276	258
	109		135		14		239	
	121				8			
156	136	141	174	196	8	8	218	215
	166		218		10		212	
	121				7			
Solvent control	110	113	91	81	7	8	182	174
	123				7			
	121		9					
	94		8					
	117		9					
2-AA 5.0 µg	806	872	80	74	114	108	154	156
	895		67		105		158	
	916				105			

Abbreviations:

2-AA: 2-aminoanthracene

**Table 3.** Number of revertant colonies per plate obtained with *Salmonella typhimurium* following exposure to Serine Endopeptidase (Batch No. PPA 26797) in the absence of metabolic activation in the treat and plate assay.

**1. experiment. Without S9 Mix**

Test Substance Concentration $\mu\text{g}$ per mL	Number of revertants (number of colonies/plate) Frame-shift mutation type							
	TA98				TA1537			
	Revertants <sup>1)</sup>		Viable cells <sup>2)</sup>		Revertants <sup>1)</sup>		Viable cells <sup>2)</sup>	
	Single plates	Mean	Single plates	Mean	Single plates	Mean	Single plates	Mean
5000	25	28	170	155	8	9	119	122
	25		139		7		125	
	33				11			
2500	33	24	161	166	9	7	96	91
	16		171		5		85	
	22				8			
1250	22	21	188	165	11	9	99	83
	21		142		11		67	
	20				6			
625	33	25	172	167	6	9	108	108
	20		161		11		108	
	23				10			
313	13	17	155	176	15	10	112	101
	18		197		11		90	
	20				3			
156	13	18	136	144	10	9	129	131
	17		152		10		132	
	23				7			
Solvent control	18	23	156	168	11	9	120	114
	26				5			
	23				3			
	27				12			
	20				15			
2-NF 20.0 $\mu\text{g}$	1173	1167	110	107				
	1095		103					
	1234							
9-AA 2.0 $\mu\text{g}$					783	744	76	71
					724		66	
					726			

Abbreviations:

2NF: 2-nitrofluorene  
9-AA: 2-aminoacridine

**Table 4.** Number of revertant colonies per plate obtained with *Salmonella typhimurium* following exposure to Serine Endopeptidase (Batch No. PPA 26797) in the presence of metabolic activation in the treat and plate assay.

**1. experiment. With S9 Mix**

Test Substance Concentration $\mu\text{g}$ per mL	Number of revertants (number of colonies/plate) Frame-shift mutation type							
	TA98				TA1537			
	Revertants <sup>1)</sup>		Viable cells <sup>2)</sup>		Revertants <sup>1)</sup>		Viable cells <sup>2)</sup>	
	Single plates	Mean	Single plates	Mean	Single plates	Mean	Single plates	Mean
5000	22	23	209	218	6	7	119	119
	24		227		9		119	
	23				5			
2500	18	18	182	183	9	7	171	173
	19		184		6		175	
	18				5			
1250	16	16	174	175	4	7	120	127
	16		175		9		133	
	17				7			
625	22	22	153	145	8	7	125	125
	24		137		5		125	
	20				7			
313	18	22	276	268	4	5	107	118
	26		260		3		129	
	23				7			
156	24	19	208	184	3	4	141	146
	20		159		6		150	
	14				4			
Solvent control	33	30	131	141	8	7	55	49
	33				7			
	27				6			
	32				7			
	26				7			
2-AA 5.0 $\mu\text{g}$	2045	2087	120	114	118	123	32	43
	2088		108		121		53	
	2127				129			

Abbreviations:

2-AA: 2-aminoanthracene

**Table 5.** Number of revertant colonies per plate obtained with *E.coli* WP2uvrApKM101 following exposure to Serine Endopeptidase (Batch No. PPA 26797) in the absence and presence of metabolic activation in the treat and plate assay.

**1. experiment. Without and with S9 Mix**

Test Substance Concentration µg per mL	Number of revertants (number of colonies/plate) Base-pair substitution type							
	E.Coli without S9				E.Coli with S9			
	Revertants <sup>1)</sup>		Viable cells <sup>2)</sup>		Revertants <sup>1)</sup>		Viable cells <sup>2)</sup>	
	Single plates	Mean	Single plates	Mean	Single plates	Mean	Single plates	Mean
5000	168		296		271		415	
	197	184	189	243	227	227	446	431
	186				182			
2500	179		324		250		297	
	197	197	303	314	245	241	249	273
	215				229			
1250	190		282		248		301	
	213	208	265	274	240	252	332	317
	220				267			
625	195		244		274		285	
	180	191	227	236	240	257	281	283
	199				256			
313	238		194		263		259	
	180	200	186	190	250	264	275	267
	182				280			
156	226		197		297		285	
	184	198	194	196	278	264	301	293
	185				216			
Solvent control	183				308			
	186		178		328		337	
	206	193	222	200	305	319	325	331
	195				340			
	196				313			
MNNG 7.5 µg	648		189					
	733	692	185	187				
	694							
2-AA 20.0 µg					1819		266	
					1797	1803	249	258
					1792			

**Abbreviations:**

MNNG: N-Methyl-N'-nitro-N-nitrosoguanidine

2-AA: 2-aminoanthracene

**Table 6.** Number of revertant colonies per plate obtained with *Salmonella typhimurium* following exposure to Serine Endopeptidase (Batch No. PPA 26797) in the absence of metabolic activation in the treat and plate assay.

**2. experiment. Without S9 Mix**

Test Substance Concentration $\mu\text{g}$ per mL	Number of revertants (number of colonies/plate) Base-pair substitution type								
	TA100				TA1535				
	Revertants <sup>1)</sup>		Viable cells <sup>2)</sup>		Revertants <sup>1)</sup>		Viable cells <sup>2)</sup>		
	Single plates	Mean	Single plates	Mean	Single plates	Mean	Single plates	Mean	
5000	112	115	36	39	6	6	55	58	
	106		41		6		61		
	128				6				
2500	101	105	34	37	11	9	60	54	
	111		39		5		47		
	103				12				
1250	179	167	104	101	10	8	123	136	
	148		98		10		148		
	173				4				
625	141	142	86	90	13	11	124	119	
	126		93		8		113		
	159				11				
313	129	136	88	96	9	9	136	132	
	147		103		6		128		
	133				11				
156	130	139	90	88	10	9	117	113	
	155		85		9		108		
	132				9				
Solvent control	140	132	86	81	17	11	96	107	
	112				13				
	131				12				118
	153				8				
	124				7				
MNNG 1.0 $\mu\text{g}$	2656	2637	52	65	1703	1733	109	128	
	2587		78		1802		147		
	2667				1693				

Abbreviations:

MNNG: N-Methyl-N'-nitro-N-nitrosoguanidine

**Table 7.** Number of revertant colonies per plate obtained with *Salmonella typhimurium* following exposure to Serine Endopeptidase (Batch No. PPA 26797) in the presence of metabolic activation in the treat and plate assay.

**2. experiment. With S9 Mix**

Test Substance Concentration µg per mL	Number of revertants (number of colonies/plate) Base-pair substitution type							
	TA100				TA1535			
	Revertants <sup>1)</sup>		Viable cells <sup>2)</sup>		Revertants <sup>1)</sup>		Viable cells <sup>2)</sup>	
	Single plates	Mean	Single plates	Mean	Single plates	Mean	Single plates	Mean
5000	108		25		12		37	
	115	119	17	21	15	12	44	41
	134				9			
2500	103		23		18		92	
	96	99	22	23	15	14	105	99
	97				10			
1250	216		152		8		191	
	194	204	139	146	8	9	170	181
	202				10			
625	162		163		17		184	
	205	186	136	150	13	15	189	187
	191				16			
313	186		98		19		182	
	195	195	136	117	11	15	180	181
	205				16			
156	172		167		12		234	
	174	182	166	167	11	12	234	234
	200				14			
Solvent control	156				5			
	222		72		15		189	
	172	172	97	85	7	11	174	182
	152				17			
2AA 5.0 µg	158				9			
	2070		47		131		85	
	1817	1907	48	48	141	147	79	82
	1834				168			

Abbreviations:

2-AA: 2-aminoanthracene

**Table 8.** Number of revertant colonies per plate obtained with *Salmonella typhimurium* following exposure to Serine Endopeptidase (Batch No. PPA 26797) in the absence of metabolic activation in the treat and plate assay.

**2. experiment. Without S9 Mix**

Test Substance Concentration $\mu\text{g}$ per mL	Number of revertants (number of colonies/plate) Frame-shift mutation type							
	TA98				TA1537			
	Revertants <sup>1)</sup>		Viable cells <sup>2)</sup>		Revertants <sup>1)</sup>		Viable cells <sup>2)</sup>	
	Single plates	Mean	Single plates	Mean	Single plates	Mean	Single plates	Mean
5000	21	22	198	186	15	12	102	106
	23		173		11		109	
	23				9			
2500	27	23	195	192	8	9	162	167
	20		188		9		172	
	21				9			
1250	11	16	137	136	9	7	107	111
	15		134		6		115	
	23				7			
625	22	19	184	187	9	9	118	116
	16		190		7		114	
	20				11			
313	11	14	182	181	15	9	102	106
	14		180		7		110	
	17				6			
156	22	22	196	180	5	7	123	112
	21		163		9		101	
	22				6			
Solvent control	16	19	150	164	9	9	155	143
	21				8			
	22				5			
	20				11			
	14				12			
2-NF 20.0 $\mu\text{g}$	937	998	188	180				
	1018		171					
	1039							
9-AA 2.0 $\mu\text{g}$					588	736	117	114
					1012		111	
					607			

Abbreviations:

2NF: 2-nitrofluorene  
9-AA: 2-aminoacridine

**Table 9.** Number of revertant colonies per plate obtained with *Salmonella typhimurium* following exposure to Serine Endopeptidase (Batch No. PPA 26797) in the presence of metabolic activation in the treat and plate assay.

**2. experiment. With S9 Mix**

Test Substance Concentration µg per mL	Number of revertants (number of colonies/plate) Frame-shift mutation type							
	TA98				TA1537			
	Revertants <sup>1)</sup>		Viable cells <sup>2)</sup>		Revertants <sup>1)</sup>		Viable cells <sup>2)</sup>	
	Single plates	Mean	Single plates	Mean	Single plates	Mean	Single plates	Mean
5000	23	24	87	98	11	10	12	12
	25		108		9		12	
	23				10		12	
2500	20	21	272	266	12	13	64	60
	22		259		16		55	
	20				12			
1250	22	20	245	253	20	17	142	125
	20		260		14		107	
	18				17			
625	29	28	220	216	11	12	145	140
	31		211		12		135	
	23				13			
313	25	22	230	225	18	21	119	117
	23		220		25		114	
	18				20			
156	27	25	202	182	13	16	120	126
	26		161		16		132	
	22				18			
Solvent control	42	28	183	184	12	13	75	67
	22				16			
	27				11			
	29				13			
	21				15			
2AA 5.0 µg	2934	2998	99	108	167	170	71	67
	2973		117		161		63	
	3086				183			

Abbreviations:

2-AA: 2-aminoanthracene

**Table 10.** Number of revertant colonies per plate obtained with *E.coli* WP2uvrApKM101 following exposure to Serine Endopeptidase (Batch No. PPA 26797) in the absence and presence of metabolic activation in the treat and plate assay.

**2. experiment. Without and with S9 Mix**

Test Substance Concentration µg per mL	Number of revertants (number of colonies/plate) Base-pair substitution type							
	E.Coli without S9				E.Coli with S9			
	Revertants <sup>1)</sup>		Viable cells <sup>2)</sup>		Revertants <sup>1)</sup>		Viable cells <sup>2)</sup>	
	Single plates	Mean	Single plates	Mean	Single plates	Mean	Single plates	Mean
5000	202		321		279		435	
	201	194	316	319	255	272	467	451
	180				283			
2500	207		287		286		351	
	190	199	276	282	305	290	325	338
	199				278			
1250	151		258		286		253	
	182	169	269	264	281	281	260	257
	174				275			
625	188		251		302		436	
	177	179	243	247	315	299	377	407
	173				280			
313	211		223		309		297	
	193	200	202	213	325	309	275	286
	197				293			
156	190		251		276		278	
	153	180	211	231	301	287	277	278
	196				285			
Solvent control	170				358			
	190		174		289		314	314
	185	178	164	169	318	310	314	
	190				334			
	153				250			
MNNG 7.5 µg	903		159					
	933	953	155	157				
	1023							
2-AA 20.0 µg					1639		267	
					1666	1611	267	267
					1528			

**Abbreviations:**

MNNG: N-Methyl-N'-nitro-N-nitrosoguanidine

2-AA: 2-aminoanthracene

## APPENDIX 1

### Historical control data

**Negative control** (purified water) ranges for *S. typhimurium* strains and *E.coli* WP2uvrApKM101 obtained in the treat and plate assay (SOP: TOX-SM-0808 and TOX-SM-0809)

Strain	S9	Number of determinations	Mean number of revertants per plate	SD	Range *)	
					lower	upper
TA1535	÷	12	7.6	5.9	3	23
	+	12	6.3	3.0	2	10
TA100	÷	13	103.2	33.0	69	182
	+	13	123.6	25.0	74	174
TA1537	÷	18	9.9	3.3	4	18
	+	13	14.3	4.0	7	28
TA98	÷	14	18.9	5.0	12	27
	+	13	22.4	5.7	12	33
WP2 uvrA pKM101	÷	10	203.3	33.4	144	253
	+	11	249.2	60.7	141	339

The above are pooled data from a number of independent determinations selected from studies conducted over the period January 2006 to March 2007, for which the correct strain and assay functioning was considered to have been confirmed. Only determinations, which were obviously vitiated by errors, have been omitted. The exception to this was TA1537 without S9 and the WP2uvrApKM101 with S9, which, due to more limited amount of data, was selected from the period March 2005-March 2007 and May 2004-March 2007, respectively.

\*) Ranges stated are the maximum and minimum mean spontaneous revertant counts from the data sets sampled.

## APPENDIX 2

### Historical control data

**Positive control ranges** for *S. typhimurium* strains and *E.coli* WP2uvrApKM101 obtained in the treat and plate assay (SOP: TOX-SM-0808 and TOX-SM-0809)

Strain	S9	Number of determinations	Chemical	Mean number of revertants per plate	SD	Range **)	
						lower	upper
TA1535	÷	12	MNNG 1 µg/mL	4147	1366	562	5744
	+	12	2-AA 5 µg/mL	144	36	77	195
TA100	÷	13	MNNG 1 µg/mL	3432	905	1293	4516
	+	13	2-AA 5 µg/mL	1209	493	458	2080
TA1537	÷	18	9-AA 2 µg/mL	1229	695	333	3167
	+	13	2-AA 5 µg/mL	136	50	62	214
TA98	÷	14	2-NF 20 µg/mL	816	363	229	1902
	+	13	2-AA 5 µg/mL	1757	517	805	2668
WP2 uvrA pKM101	÷	4	MNNG *) 7.5 µg/mL	1085	412	692	1663
	+	10	2-AA 20 µg/mL	1272	258	917	1681

The above are pooled data from a number of independent determinations selected from studies conducted over the period January 2006 to March 2007, for which the correct strain and assay functioning was considered to have been confirmed. Only determinations, which were obviously vitiated by errors, have been omitted. The exception to this was TA1537 without S9 and the WP2uvrApKM101 with S9, which, due to more limited amount of data within this period, was selected from March 2005-March 2007 and May 2004-March 2007, respectively.

\*) The number of studies in the past years with the specific dose applied in this study is limited due to a change of our standard control mutagen. The data was sampled from studies conducted most recently in 2007.

\*\*) Ranges stated are the maximum and minimum mean revertant colony counts from the data sets sampled.

#### Abbreviations:

2NF: 2-nitrofluorene  
 MNNG: N-Methyl-N'-nitro-N-nitrosoguanidine  
 2-AA: 2-aminoanthracene  
 9-AA: 2-aminoacridine

### APPENDIX 3

#### PREPARATION OF MEDIA

##### 1. Top-agar - histidine-deficient soft agar

Agar, Merck	0.6 g
NaCl	0.5 g
Distilled water to	100 ml

The medium was autoclaved for 15 minutes at 121°C. After cooling to about 60°C, 10 ml of a sterile aqueous solution of 0.5 mM biotin - 0.5 mM histidine was added aseptically.

##### 2. Nutrient broth - histidine-rich broth

Difco nutrient broth	8 g
NaCl	5 g
Distilled water to	1 litre

The medium was autoclaved for 15 minutes at 121°C.

##### 3. Nutrient agar - histidine-rich agar medium

Agar, Merck	15 g
Oxoid nutrient broth No. 2	25 g
Distilled water to	1 litre

The medium was autoclaved for 15 minutes at 121°C.

##### 4. Minimal medium

This was Vogel-Bonner minimal "E" medium with 2% glucose, prepared as follows :

###### Solution A (Vogel-Bonner medium E, 20X)

MgSO <sub>4</sub> 7H <sub>2</sub> O	4 g
Citric acid, monohydrate	40 g
K <sub>2</sub> HPO <sub>4</sub>	200 g
NaH <sub>2</sub> NH <sub>4</sub> 4H <sub>2</sub> O	70 g
Distilled water to	1000 ml

The solution was sterilized by filtration.

###### Solution B (40% glucose)

Glucose	40 g
Distilled water to	100 ml

This solution was sterilized by filtration.

###### Solution C (Agar base)

Agar, Merck	16.7 g
Distilled water to	1000 ml

Solution C was autoclaved for 15 minutes at 121°C. After cooling to 60°C, 450 ml of solution C was aseptically added 25 ml solution A and 25 ml solution B.

**AMENDMENT TO FINAL REPORT**

Study Number:	1974/62	
Amendment Issue Date:	July 2009	
Amendment Number:	1	
Study Title:	Serine Endopeptidase (PPA 26797): Induction of chromosome aberrations in cultured human peripheral blood lymphocytes	
Authorised By:	[REDACTED]	01 July 09
	Study Director	Date
Quality Assurance Audit:	[REDACTED]	01 July 2009
	QA Representative	Date
Distribution:	Study Director, QA, Sponsor	
This amendment to final report contains 48 pages including this one.		
Documentation		
Section(s) amended and explanation for the change		
<p>For confidentiality reasons the internal Novozymes name, used in the development phase and toxicological studies of the enzyme, has been taken out of the present study report and has been replaced by the generic enzyme name – Serine Endopeptidase, followed by an unambiguous identification number of the test batch (PPA 26797). The sample identification number of the test batch, PPA 26797, is exactly the same as used in the original report.</p> <p>The title page and headers of all pages has been altered to reflect the fact that this report is now Amended Final Report 1, and the date of the report issued has been changed from September 2007 to July 2009.</p> <p>Page 47 The Certificate of Analysis included as an appendix to the report has been changed to a new version</p> <p>The QA page has been amended to include the Report Amendment Review and the associated dates of 25 June 2009.</p> <p>All signature pages have been re-signed with reference to the amended final report.</p>		

# Amended Final Report 1

Study Title	Serine Endopeptidase (PPA 26797): Induction of chromosome aberrations in cultured human peripheral blood lymphocytes
Test Article	Serine Endopeptidase (PPA 26797)
Author	██████████
Sponsor	Novozymes A/S Safety and Toxicology Krogshoejvej 36 DK-2880 Bagsvaerd DENMARK
Study Monitor	██████████
Test Facility	Covance Laboratories Ltd Otley Road, Harrogate North Yorkshire HG3 1PY ENGLAND
Covance Study Number	1974/62
Covance Report Number	1974/62-D6172
Novozymes Reference Number	20076030
Report Issued	July 2009
Page Number	1 of 47

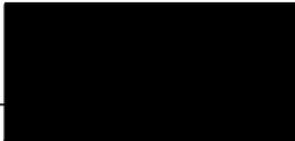
**STUDY DIRECTOR AUTHENTICATION  
AND GLP COMPLIANCE STATEMENT**

**Serine Endopeptidase (PPA 26797): Induction of chromosome aberrations in  
cultured human peripheral blood lymphocytes**

I, the undersigned, hereby declare that the work was performed under my supervision and that the findings provide a true and accurate record of the results obtained.

The study was performed in accordance with the agreed protocol and with Covance Laboratories Limited Standard Operating Procedures, unless otherwise stated, and the study objectives were achieved.

The study was conducted in compliance with the United Kingdom Good Laboratory Practice Regulations 1999, Statutory Instrument No. 3106 as amended by the Good Laboratory Practice (Codification Amendments Etc.) Regulations 2004 and the OECD Principles on Good Laboratory Practice (revised 1997, issued January 1998) ENV/MC/CHEM (98) 17.

  \_\_\_\_\_  
Study Director

\_\_\_\_\_ 01 July 09  
Date

## QUALITY ASSURANCE STATEMENT

### Serine Endopeptidase (PPA 26797): Induction of chromosome aberrations in cultured human peripheral blood lymphocytes

This study has been reviewed by the Quality Assurance Unit of Covance Laboratories Ltd. and the report accurately reflects the raw data. The following inspections were conducted and findings reported to the study director (SD) and associated management.

Critical procedures, which are performed routinely in an operational area, may be audited as part of a "process" inspection program. This can be in addition to phases scheduled on an individual study basis. Selected process inspections conducted and considered applicable to this study are included below.

In addition to the inspection programmes detailed below, a facility inspection programme is also operated. Details of this programme, which covers all areas of the facility annually (at a minimum), are set out in standard operating procedures.

Inspection Dates		Phase	Date Reported to SD and SD Management
From	To		
29 May 2007	29 May 2007	Protocol Review	29 May 2007
09 Aug 2007	09 Aug 2007	Protocol Amendment Review	09 Aug 2007
16 Aug 2007	24 Aug 2007	Draft Report and Data Review	24 Aug 2007
13 Sep 2007	13 Sep 2007	Final Report Review	13 Sep 2007
25 Jun 2009	25 Jun 2009	Report Amendment Review	25 Jun 2009

Inspection Dates		Phase	Date Reported to SD and SD Management
From	To		
05 Jun 2007	05 Jun 2007	Dose Preparation	05 Jun 2007
07 Jun 2007	07 Jun 2007	Test Article Dilutions	07 Jun 2007
11 Jun 2007	11 Jun 2007	Slide Decoding	12 Jun 2007
13 Jun 2007	13 Jun 2007	Slide Staining	13 Jun 2007
20 Jun 2007	20 Jun 2007	Slide Analysis	20 Jun 2007
20 Jun 2007	20 Jun 2007	Dose Preparation	20 Jun 2007
27 Jun 2007	27 Jun 2007	Historical Control Ranges	27 Jun 2007
11 Jul 2007	11 Jul 2007	S9 Mix Preparations	11 Jul 2007
11 Jul 2007	11 Jul 2007	Slide Analysis	11 Jul 2007
18 Jul 2007	18 Jul 2007	Dose Preparation	18 Jul 2007



Quality Assurance Unit

01 July 2009  
Date

**REVIEWING SCIENTIST'S STATEMENT**

**Serine Endopeptidase (PPA 26797): Induction of chromosome aberrations in cultured human peripheral blood lymphocytes**

I, the undersigned, hereby declare that I have reviewed this report in conjunction with the Study Director and that the interpretation and presentation of the data in the report are consistent with the results obtained.



Scientist

1 July 2009  
Date

**RESPONSIBLE PERSONNEL**

**Serine Endopeptidase (PPA 26797): Induction of chromosome aberrations in cultured human peripheral blood lymphocytes**

The following personnel were responsible for key elements of the study:

Study Director  
Study Supervisor



## **ARCHIVE STATEMENT**

### **Serine Endopeptidase (PPA 26797): Induction of chromosome aberrations in cultured human peripheral blood lymphocytes**

All primary data, or authenticated copies thereof, slides and the final report will be retained in the Covance Laboratories Limited archives for one year after issue of the final report. At the end of this specified archive period the Sponsor will be contacted to determine whether the data should be returned, retained or destroyed on their behalf. Sponsors will be notified of the financial implications of each of these options at that time.

Specimens or samples requiring frozen storage at Covance are specifically excluded from the above. These will be retained for as long as the material permits further evaluation, up to three months after issue of the draft report. At this time, the Sponsor will be contacted to determine whether samples should be returned, retained or destroyed on their behalf. Any financial implications of these options will also be notified at this time. Samples will not be destroyed without prior approval of the Study Director.

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## SUMMARY

Serine Endopeptidase (PPA 26797) was tested in an *in vitro* cytogenetics assay using duplicate human lymphocyte cultures prepared from the pooled blood of three male donors in two independent experiments. Treatments covering a broad range of concentrations, separated by narrow intervals, were performed both in the absence and presence of metabolic activation (S-9). The test article was formulated in sterile water for injection (purified water) and the highest concentration, 5000 µg/mL, is an acceptable maximum concentration for *in vitro* chromosome aberration studies according to current regulatory guidelines.

In Experiment 1, treatment in the absence and presence of S-9 was for 3 hours followed by a 17 hour recovery period prior to harvest (3+17). The S-9 used was prepared from a rat liver post-mitochondrial fraction (S-9) from Aroclor 1254 induced animals. The test article concentrations for chromosome analysis were selected by evaluating the effect of Serine Endopeptidase (PPA 26797) on mitotic index. Chromosome aberrations were analysed at three concentrations (see below).

S-9	Treatment + recovery (hours)	Vehicle control	Concentration (µg/mL) Serine Endopeptidase (PPA 26797)	Percentage Cytotoxicity †
-	3+17	0 <sup>a</sup>	1582, 2813, 5000	42%
+	3+17	0 <sup>a</sup>	1582, 2813, 5000	11%

<sup>a</sup> Vehicle control was purified water only

† At highest analysed concentration

In Experiment 2, treatment in the absence of S-9 was continuous for 20 hours (20+0). Treatment in the presence of S-9 was for 3 hours only followed by a 17 hour recovery period prior to harvest (3+17). Chromosome aberrations were analysed at three concentrations (see below).

S-9	Treatment + recovery (hours)	Vehicle control	Concentration (µg/mL) Serine Endopeptidase (PPA 26797)	Percentage Cytotoxicity †
-	20+0	0 <sup>a</sup>	1311, 2048, 4000	60%
+	3+17	0 <sup>a</sup>	2048, 3200, 5000	25%

<sup>a</sup> Vehicle control was purified water only

† At highest analysed concentration

Appropriate negative (vehicle) control cultures were included in the test system in both experiments under each treatment condition. The proportion of cells with structural aberrations in these cultures fell within historical vehicle control ranges. 4-Nitroquinoline 1-oxide (NQO) and cyclophosphamide (CPA) were employed as positive control chemicals in the absence and presence of rat liver S-9 respectively. Cells receiving these were sampled in each experiment, 20 hours after the start of treatment; both compounds induced statistically significant increases in the proportion of cells with structural aberrations.

### **Experiment 1**

Treatment of cultures with Serine Endopeptidase (PPA 26797) in the absence and the presence of metabolic activation (S-9) resulted in frequencies of cells with structural chromosome aberrations which were similar to those observed in concurrent vehicle control cultures for all concentrations analysed. The aberrant cell frequency of all Serine Endopeptidase (PPA 26797) treated cultures fell within current historical vehicle control (normal) ranges.

### **Experiment 2**

Treatment of cultures with Serine Endopeptidase (PPA 26797) in the presence of S-9 in Experiment 2 resulted in frequencies of cells with structural chromosome aberrations which were similar to those observed in concurrent vehicle control cultures for the majority of concentrations analysed. With the exception of a marginal increase in a single culture at 5000 µg/mL, the aberrant cell frequency of all Serine Endopeptidase (PPA 26797) cultures fell within normal ranges. As such, the marginal isolated increase was not considered of biological importance.

Continuous 20+0 hour treatment of cultures with Serine Endopeptidase (PPA 26797) in the absence of S-9 resulted in frequencies of cells with structural chromosome aberrations which were similar to those observed in concurrent vehicle control cultures for all concentrations analysed. The aberrant cell frequency of all Serine Endopeptidase (PPA 26797) treated cultures fell within normal ranges.

### **Numerical aberrations**

No increases in the frequency of cells with numerical aberrations, which exceeded the historical negative control range were observed in the majority of cultures treated with Serine Endopeptidase (PPA 26797) in the absence and presence of S-9 (all experiments).

It is concluded that Serine Endopeptidase (PPA 26797) did not induce chromosome aberrations in cultured human peripheral blood lymphocytes when tested either to 5000 µg/mL (an acceptable maximum concentration for chromosome aberration studies according to current regulatory guidelines), or, to a regulatory acceptable limit of cytotoxicity. Treatments were conducted in both the absence and presence of a rat liver metabolic activation system (S-9).

## INTRODUCTION

Chromosome defects are recognised as the basis of a number of human genetic diseases (1).

The purpose of the *in vitro* chromosome aberration test is to identify agents that cause structural chromosome aberrations in cultured mammalian cells. No one assay has been extensively evaluated on the same compounds in several laboratories but there is a large database on the use of chromosomal assays for screening purposes (2). The use of human peripheral blood lymphocytes is recommended because the cells are only used in short-term culture and maintain a stable karyotype (3). Experiments with these cells can also be performed in conjunction with a rat liver metabolising system (S-9) as for short incubation periods, no toxicity is induced by the liver homogenate itself. Increases in numerical chromosome aberrations can be detected but the assay is not specifically designed to evaluate potential to induce aneuploidy or polyploidy.

In the first instance, cells are exposed to the test article both in the absence and presence of rat liver S-9 (from rats induced with Aroclor 1254) for 3 hours and sampled at 20 hours after the beginning of treatment. This is equivalent to approximately one and a half times the average generation time of cultured lymphocytes from the panel of donors used in this laboratory. As a number of chemicals have been reported as only exerting positive effects following prolonged treatment (4, 5, 6), provision was made for a second experiment involving continuous treatment for 20 hours in the absence of S-9. This, and a repeat treatment in the presence of S-9, using a different spacing of test article concentrations, was performed in the event of a negative result in Experiment 1 (6).

Some chemicals (e.g. nucleoside/tide analogs or nitrosamides) may be more readily detected by treatment/sampling times longer than 20 hours (6). Provision was therefore made for sampling at later times for test articles known to belong to such chemical classes. In this study, further sampling times were not required.

The objective of this study was to evaluate the clastogenic potential of Serine Endopeptidase (PPA 26797) by examining its effects on the chromosomes of cultured human peripheral blood lymphocytes treated in the absence and presence of a rat liver metabolising system (S-9). The test methodology in this study is in accordance with current literature and complies with the following regulatory guidelines: OECD Guideline 473 (1997) and the ICH Tripartite Harmonised Guideline on Genotoxicity: Specific Aspects of Regulatory Tests (1995) (6, 7, 8).

This study was performed according to the protocol and one amendment.

The study was initiated on 21 May 2007. Experimental work started on 31 May 2007 and was completed on 20 July 2007. The study completion date is considered to be the date the Study Director signs the final report.

## MATERIALS

### Test article

Serine Endopeptidase (PPA 26797), batch number PPA 26797, was a brown liquid. It was received on 16 May 2007 and stored at -20°C in the dark. Purity (activity) was stated as 54600 PROT/g, but for the purposes of this study was considered as 100%. The expiry date was given as 29 March 2017. The certificate of analysis provided by the Sponsor, is presented in Appendix 7. The test article information and certificate of analysis provided by the Sponsor are considered an adequate description of the characterisation, purity and stability of the test article. Determinations of stability and characteristics of the test article were the responsibility of the Sponsor.

Serine Endopeptidase (PPA 26797) is a high molecular weight protein (approximately 19 kDa), which was formulated in purified water to a concentration of 50 mg/mL (weighed out as received), equivalent to 5000 µg/mL final concentration following dilution into the test system (the recommended maximum for *in vitro* chromosome aberration studies according to current regulatory guidelines).

The test article stock solutions were not membrane filter-sterilised prior to use and subsequent dilutions were made using sterile purified water. The test article solutions were protected from light and used within 3 hours of initial formulation as follows:

Experiment 1 Concentration of treatment solution (mg/mL)	Final concentration (µg/mL)	Hours treatment + hours recovery	
		3+17 -S-9	3+17 +S-9
2.112	211.2	✓	✓
2.816	281.6	✓	✓
3.754	375.4	✓	✓
5.006	500.6	✓	✓
6.674	667.4	✓	✓
8.899	889.9	✓	✓
11.87	1187	✓	✓
15.82	1582	✓	✓
21.09	2109	✓	✓
28.13	2813	✓	✓
37.50	3750	✓	✓
50.00	5000	✓	✓

✓ Indicates concentration tested

Experiment 2 Concentration of treatment solution (mg/mL)	Final concentration (µg/mL)	Hours treatment + hours recovery	
		20+0 -S-9	3+17 +S-9
6.711	671.1	✓	
8.389	838.9	✓	
10.49	1049	✓	
13.11	1311	✓	
16.38	1638	✓	✓
20.48	2048	✓	✓
25.60	2560	✓	✓
32.00	3200	✓	✓
40.00	4000	✓	✓
50.00	5000	✓	✓

✓ Indicates concentration tested

It should be noted that an initial trial of Experiment 2 was aborted due to low vehicle control mitotic indices, indicating poor cellular proliferation. Data from this initial trial has not been further reported.

Changes in osmolality of more than 50 mOsm/kg, and fluctuations in pH of more than one unit, can give rise to chromosome aberrations (9, 10).

Measurements on post-treatment media from Experiment 1 in the absence and presence of S-9 indicated that the test article had no marked effect on osmolality (greater than a shift of 50 mOsm/kg) or pH (shift of greater than 1 pH unit) as compared to concurrent vehicle controls.

### Controls

Sterile purified water was added to cultures designated as negative controls as described in the methods section of this report. The positive control chemicals were dissolved in sterile anhydrous analytical grade dimethyl sulphoxide (DMSO), frozen down and thawed out immediately prior to use as follows:

Chemical	Supplier	Concentration of treatment solution (mg/mL)	Final concentration (µg/mL)	S-9
4-Nitroquinoline 1-oxide (NQO)	Sigma-Aldrich Chemical Co, Poole, UK	0.250	2.50	-
		0.500	5.00	-
Cyclophosphamide (CPA)	Sigma-Aldrich Chemical Co, Poole, UK	0.625	6.25	+
		1.25	12.5	+

Cells treated with 5.00 µg NQO/mL and 12.5 µg CPA/mL gave satisfactory responses in terms of quality and quantity of mitoses and extent of chromosomal damage. These were selected for analysis.

### **Metabolic activation system**

The mammalian liver post-mitochondrial fraction (S-9) used for metabolic activation was obtained from Molecular Toxicology Incorporated, USA where it is prepared from male Sprague Dawley rats induced with Aroclor 1254. The batches of MolTox™ S-9 were stored frozen in aliquots at -80°C nominal prior to use. Each batch was checked by the manufacturer for sterility, protein content, ability to convert known promutagens to bacterial mutagens and cytochrome P-450-catalyzed enzyme activities (alkoxyresorufin-O-dealkylase activities). The quality control statements, relating to the batches of S-9 preparation used, are included in Appendix 6 of this report.

Treatment was carried out both in the absence and presence of S-9. The S-9 mix was prepared in the following way:

Glucose-6-phosphate (180 mg/mL), NADP (25 mg/mL), Potassium chloride (KCl) (150 mM) and rat liver S-9 were mixed in the ratio 1:1:1:2. For all cultures treated in the presence of S-9 an aliquot of the resulting mix was added to each cell culture to achieve the required final concentration of the test article in a total of 10 mL. The final concentration of liver homogenate in the test system was 2%.

Cultures treated in the absence of S-9 received an equivalent volume of 150 mM KCl.

### **Blood cultures**

Blood from three healthy, non-smoking male volunteers was used for each experiment of this study:

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Experiment	Donor Sex	Donor Age (years)	Donor Identity
1	Male	23, 29, 25	9402, 7100, 9932
2	Male	34, 23, 37	7325, 9402, 5553

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No volunteer was suspected of any virus infection or exposed to high levels of radiation or hazardous chemicals. The measured cell cycle time of the donors used at Covance falls within the range 13 +/- 1.5 hours. For each experiment, an appropriate volume of whole blood was drawn from the peripheral circulation into heparinised

tubes within one day of culture initiation. Blood was stored refrigerated and pooled using equal volumes from each donor prior to use.

Whole blood cultures were established in sterile disposable centrifuge tubes by placing 0.4 mL of pooled heparinised blood into 8.1 mL HEPES-buffered RPMI medium containing 20% (v/v) heat inactivated foetal calf serum and 50 µg/mL gentamycin, so that the final volume following addition of S-9 mix or KCl and the test article in its chosen vehicle is 10 mL. Phytohaemagglutinin (PHA, reagent grade) was included in the culture medium at a concentration of approximately 2% of culture to stimulate the lymphocytes to divide. Blood cultures were incubated at 37°C±1°C for approximately 48 hours and rocked continuously.

## METHODS

The test system was suitably labelled (using a colour-coded procedure) to clearly identify the study number, experiment number, treatment time, test article concentration, positive and negative controls.

Immediately prior to treatment, all positive control cultures had 0.9 mL culture medium added to give a final pre-treatment volume of 9.4 mL.

S-9 mix or KCl (0.5 mL per culture) was added appropriately. Quadruplicate cultures (A, B, C and D) were treated with the vehicle and duplicate cultures (A, B) treated with the test article at appropriate concentrations (1 mL per culture). Additional duplicate cultures were treated with 0.1 mL of the positive control chemicals.

Experiment 1 comprised a 3 hour treatment + and -S-9 followed by a 17 hour recovery period (3+17). Experiment 2 comprised a 3 hour treatment +S-9 followed by a 17 hour recovery period (3+17) and a 20 hour continuous treatment -S-9 (20+0). The final culture volume was 10 mL. Cultures were incubated at 37°C ± 1°C for the designated exposure time.

This scheme is illustrated below:

Treatment	S-9	Number of cultures	
		3+17*	20+0*
Experiment 1			
Negative control	-	4	
	+	4	
Test article	-	2	
(all concentrations)	+	2	
Positive controls	-	2	
(all concentrations)	+	2	
Experiment 2			
Negative control	-		4
	+	4	
Test article	-		2
(concentrations as appropriate)	+	2	
Positive controls	-		2
(all concentrations)	+	2	

\* Hours treatment + hours recovery

Cultures receiving continuous treatment retained medium through to harvest. Pulse treatments were for 3 hours only. Cells were then pelleted (approximately 300 x 'g',

10 minutes), washed twice with sterile saline (pre-warmed at approximately 37°C) and resuspended in fresh pre-warmed medium containing foetal calf serum and gentamycin. Pulse treatment cultures were incubated for a further 17 hours before harvesting.

### Summary of treatment conditions

Treatment	S-9	Duration of treatment (hours)	Harvest time (hours after start of treatment)
Continuous	20+0	-	20
Pulse	3+17	+	3
	3+17	-	3

### Harvesting

Approximately 2 hours prior to harvest, colchicine was added to give a final concentration of approximately 1 µg/mL to arrest dividing cells in metaphase. At the defined sampling time cultures were centrifuged at approximately 300 x 'g' for 10 minutes; the supernatant was carefully removed and cells were resuspended in 4 mL pre-warmed hypotonic (0.075 M) KCl and incubated at 37°C±1°C for 15 minutes to allow cell swelling to occur. Cells were then fixed by dropping the KCl suspension into fresh, cold methanol/glacial acetic acid (3:1, v/v). The fixative was changed by centrifugation (approximately 300 x 'g', 10 minutes) and resuspension. This procedure was repeated several times (centrifuging at approximately 1250 x 'g', two to three minutes) until the cell pellets were clean.

### Slide preparation

Lymphocytes were kept in fixative at 1-10°C before slides were made but slides were not made on the day of harvest to ensure that cells were adequately fixed. Cells were centrifuged and resuspended in a minimal amount of fresh fixative (if required) to give a milky suspension. Several drops of 45% (v/v) aqueous acetic acid were added to each suspension to enhance chromosome spreading, and several drops of suspension were transferred on to clean microscope slides labelled with the appropriate study details. Slides were flamed, as necessary, to improve quality. After the slides had dried the cells were stained for 5 minutes in filtered 4% (v/v) Giemsa in pH 6.8 buffer. The slides were rinsed, dried and mounted with coverslips.

### **Selection of concentrations for chromosome analysis**

Slides were examined, uncoded, for MI to determine whether chemically induced mitotic inhibition had occurred.

The Mitotic Index (MI) is a measure of the proliferative state of the culture at a particular moment in time and was calculated as follows:

$$MI = \frac{\text{number of cells in mitosis}}{\text{Total number of cells observed}} \times 100$$

The highest concentration for chromosome analysis from cultures sampled at 20 hours should be one at which at least (or approximately) 50% mitotic inhibition has occurred or should be the highest concentration tested.

Analysis of slides from highly cytotoxic concentrations was avoided. Slides from the highest selected concentration and two lower concentrations were taken for microscopic analysis, such that (where appropriate) a range of mitotic inhibition from maximum to little or none was covered.

For each treatment regime, two vehicle control cultures were analysed for chromosome aberrations. Slides from the remaining vehicle control cultures were only to be analysed if considered necessary, for example, to help resolve an equivocal result. In this study analysis of additional vehicle control cultures was not required.

A single positive control concentration, which gave satisfactory responses in terms of quality and quantity of mitoses and extent of chromosomal damage, was analysed.

Mitotic index data and the results of concentration selection are presented in the results section of this report.

### **Slide analysis**

Slides from NQO and CPA positive control treatments were checked to ensure that the system was operating satisfactorily. All slides for analysis were coded using randomly generated letters by an individual not connected with the scoring of the slides. Labels with only the study number, experiment number, the sex of the donor and the code were used to cover treatment details on the slides.

Where appropriate, one hundred metaphases from each code were analysed for chromosome aberrations. Where 10 cells with structural aberrations (excluding gaps) were noted on a slide, analysis may have been terminated. Only cells with 44 to 46 chromosomes were considered acceptable for analysis. Any cell with more than 46 chromosomes (that is, polyploid, hyperdiploid or endoreduplicated cells) observed during this evaluation was noted and recorded separately. Structural aberrations were classified according to the ISCN scheme (11) as detailed in Appendix 2.

Under this scheme, a gap is defined as a discontinuity less than the width of the chromatid with no evidence of displacement of the fragment and a deletion is defined as a discontinuity greater than the width of the chromatid and/or evidence of displacement of the fragment. Observations (summarised in Appendix 3 and Appendix 4) were recorded on raw data sheets with the microscope stage co-ordinates of the first five cells, all aberrant cells and the last cell scored.

Slide analysis was performed by competent analysts trained in the applicable Covance Laboratories Harrogate (CLEH) standard operating procedures. Although physically located remote from the CLEH facility, all analysts were subject to CLEH management and GLP control systems (including QA inspection). All slides and raw data generated have been returned to CLEH for archiving.

## **Analysis of results**

### **Treatment of data**

After completion of scoring and decoding of slides the numbers of aberrant cells in each culture were categorised as follows:

1. cells with structural aberrations including gaps
2. cells with structural aberrations excluding gaps
3. polyploid, endoreduplicated or hyperdiploid cells.

The totals for category 2 in negative control cultures were compared with the current laboratory historical negative control (normal) ranges to determine whether the assay was acceptable or not (see Acceptance criteria). The totals for category 2 in test article treated cultures were also compared with normal ranges. The statistical significance of increases in the percentage of cells with structural aberrations for any data set was only to be taken into consideration if the frequency of aberrant cells in both replicate cultures at one or more concentrations

exceeds the normal range. The statistical method used would be Fisher's exact test (12). Probability values of  $p \leq 0.05$  were accepted as significant. The proportions of cells in categories 1 and 3 were also examined in relation to normal ranges and may be analysed by Fisher's exact test.

The proportions of aberrant cells in each replicate were also used to establish acceptable heterogeneity between replicates by means of a binomial dispersion test (12). Probability values of  $p \leq 0.05$  were to be accepted as significant.

### **Acceptance criteria**

The assay was considered valid if all the following criteria were met:

1. The binomial dispersion test demonstrated acceptable heterogeneity between replicate cultures.
2. The proportion of cells with structural aberrations (excluding gaps) in negative control cultures fell within the historical negative control (normal) range.
3. At least 160 cells out of an intended 200 were suitable for analysis at each concentration, unless 10 or more cells showing structural aberrations (per slide) other than gaps only were observed during analysis.
4. The positive control chemicals induced statistically significant increases in the proportion of cells with structural aberrations.

### **Evaluation criteria**

The data were evaluated as to whether exposure to the test article was associated with:

1. A proportion of cells with structural aberrations at one or more concentrations exceeded the historical negative control (normal) range in both replicate cultures
2. A statistically significant increase in the proportion of cells with structural aberrations (excluding gaps) was observed at such concentrations
3. There was a concentration-related trend in the proportion of cells with structural aberrations (excluding gaps).

The test article was to be considered positive in this assay if all of the above criteria were met.

The test article was to be considered negative in this assay if none of the above criteria were met.

Results which only partially satisfied the above criteria were to be dealt with on a case-by-case basis. Evidence of a concentration-related effect is considered useful but not essential in the evaluation of a positive result (13). Biological relevance was taken into account, for example consistency of response within and between concentrations and (where applicable) between experiments, or effects occurring only at high or very toxic concentrations, and the types and distribution of aberrations.

## RESULTS

### Selection of concentrations for cytogenetic analysis

The results of mitotic index determinations for the treatments in Experiment 1 were as follows:

Treatment (µg/mL)	3+17 hours, -S-9		Mitotic index (%)			
	A/C	B/D	MIH*	A/C	B/D	MIH*
Vehicle	8.6/6.7	7.3/6.9	-	7.3/7.4	7.7/9.0	-
211.2	NS	NS	-	NS	NS	-
281.6	NS	NS	-	NS	NS	-
375.4	NS	NS	-	NS	NS	-
500.6	NS	NS	-	NS	NS	-
667.4	NS	NS	-	NS	NS	-
889.9	NS	NS	-	NS	NS	-
1187	7.4	7.2	1	10.6	8.4	0
1582	<b>7.2</b>	<b>8.2</b>	<b>0</b>	<b>9.0</b>	<b>8.9</b>	<b>0</b>
2109	6.6	6.4	12	6.0	5.6	26
2813	<b>4.8</b>	<b>6.0</b>	<b>27</b>	<b>6.3</b>	<b>6.5</b>	<b>18</b>
3750	5.0	4.2	38	6.7	6.3	17
5000	<b>4.5</b>	<b>4.1</b>	<b>42</b>	<b>7.9</b>	<b>6.0</b>	<b>11</b>

NS = not scored

\*Mitotic inhibition (%) =  $[1 - (\text{mean MI}_T / \text{mean MI}_C)] \times 100\%$

(where T = treatment and C = negative control)

(Slides from vehicle control cultures C and D scored for mitotic index only)

A/C, B/D refers to the number of cultures treated (four [A, B, C, D] for vehicle controls and two [A, B] for test article and positive controls)

Shaded concentrations selected for analysis

The results of mitotic index determinations for the treatments in Experiment 2 were as follows:

Treatment (µg/mL)	20+0 hours, -S-9			3+17 hours, +S-9		
	A/C	B/D	MIH*	A/C	B/D	MIH*
Vehicle	8.6/6.9	10.4/8.4	-	10.7/9.2	8.9/8.2	-
671.1	NS	NS	-	NT	NT	-
838.9	NS	NS	-	NT	NT	-
1049	NS	NS	-	NT	NT	-
1311	<b>8.4</b>	<b>9.1</b>	<b>0</b>	NT	NT	-
1638	7.4	9.6	1	10.9	8.6	0
2048	<b>6.1</b>	<b>5.6</b>	<b>32</b>	<b>8.2</b>	<b>9.9</b>	<b>2</b>
2560	4.5	6.8	34	7.4	9.1	11
3200	3.1	4.0	59	<b>8.1</b>	<b>7.0</b>	<b>18</b>
4000	<b>3.4</b>	<b>3.5</b>	<b>60</b>	7.5	7.4	19
5000	2.7	3.1	66	<b>7.5</b>	<b>6.3</b>	<b>25</b>

NT = not tested NS = not scored

\*Mitotic inhibition (%) =  $[1 - (\text{mean MI}_T / \text{mean MI}_C)] \times 100\%$   
(where T = treatment and C = negative control)  
(Slides from vehicle control cultures C and D scored for mitotic index only).

A/C, B/D refers to the number of cultures treated (four [A, B, C, D] for vehicle controls and two [A, B] for test article and positive controls)

Shaded concentrations selected for analysis

## Chromosome aberration analysis

### Raw data

The raw data for the observations on the test article plus positive and negative controls are retained by Covance Laboratories Limited. A summary of the number of cells containing structural aberrations is given in Appendix 1 for each of the different treatment regimes in Experiment 1 and 2. The numbers and types of structural aberrations seen per cell are given in Appendix 3. Frequencies of cells with numerical aberrations observed are given in Appendix 4.

### Validity of study

The data in Appendix 1 and Appendix 5 confirm that:

1. the binomial dispersion test demonstrated acceptable heterogeneity between replicate cultures (Appendix 1)
2. the proportion of cells with structural aberrations (excluding gaps) in negative control cultures fell within the historical negative control (normal) range (Appendix 5)

3. at least 160 cells out of an intended 200 were suitable for analysis at each concentration, unless 10 or more cells showing structural aberrations (per slide) other than gaps only were observed during analysis (Appendix 1)
4. the positive control chemicals induced statistically significant increases in the proportion of cells with structural aberrations (Appendix 1).

## **Analysis of data**

### **Structural aberrations**

#### **Experiment 1**

Treatment of cultures with Serine Endopeptidase (PPA 26797) in the absence and the presence of metabolic activation (S-9) resulted in frequencies of cells with structural chromosome aberrations which were similar to those observed in concurrent vehicle control cultures for all concentrations analysed. The aberrant cell frequency of all Serine Endopeptidase (PPA 26797) treated cultures fell within current historical vehicle control (normal) ranges (Appendix 1 and Appendix 5).

#### **Experiment 2**

Treatment of cultures with Serine Endopeptidase (PPA 26797) in the presence of S-9 in Experiment 2 resulted in frequencies of cells with structural chromosome aberrations which were similar to those observed in concurrent vehicle control cultures for the majority of concentrations analysed. The one exception to this was for a single replicate culture at the highest concentration analysed (5000 µg/mL) which exhibited an aberrant cell frequency marginally exceeding the historical vehicle control range. However, this increase was small (4% versus a calculated historical range of 0-3%), was not observed in the replicate culture for this or in any other test article treated culture. Furthermore, there was no evidence of any increase in aberrant cell frequency following the +S-9 treatment in Experiment 1, conducted under identical treatment conditions at equivalent concentrations. As such this marginal increase was not considered of biological importance.

Continuous 20+0 hour treatment of cultures with Serine Endopeptidase (PPA 26797) in the absence of S-9 resulted in frequencies of cells with structural chromosome aberrations which were similar to those observed in concurrent vehicle control cultures for all concentrations analysed. The aberrant cell frequency of all Serine Endopeptidase (PPA 26797) treated cultures fell within current normal ranges (Appendix 1 and Appendix 5).

### **Numerical aberrations**

No increases in the frequency of cells with numerical aberrations, which exceeded the historical negative control range were observed in the majority of cultures treated with Serine Endopeptidase (PPA 26797) in the absence and presence of S-9 (both experiments). The exceptions to this were for both replicate cultures at 4000 µg/mL following 20+0 hour –S-9 treatment. However, in both instances the increase in polyploid cells observed was marginal (2% versus historical range of 0-1%) and as such was not considered of biological importance (Appendix 4 and Appendix 5).

## CONCLUSION

It is concluded that Serine Endopeptidase (PPA 26797) did not induce chromosome aberrations in cultured human peripheral blood lymphocytes when tested to 5000 µg/mL, an acceptable maximum concentration for *in vitro* chromosome aberration studies according to current regulatory guidelines, in both the absence and presence of a rat liver metabolic activation system (S-9).

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## **APPENDICES**

**Appendix 1**  
**Serine Endopeptidase (PPA 26797): cells with structural aberrations**

**Table 1**  
**3 hour treatment -S-9, 17 hour recovery (3+17), Experiment 1**  
**Donor sex: male**

Treatment (µg/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	MIH (%)*
Vehicle	A	100	3	2	
	B	100	1	0	
	Totals	200	4	2	-
1582	A	100	3	1	
	B	100	1	1	
	Totals	200	4	2	0
2813	A	100	2	0	
	B	100	0	0	
	Totals	200	2	0	27
5000	A	100	0	0	
	B	100	1	0	
	Totals	200	1	0	42
NQO, 5.00	A	78	<b>19</b>	<b>18</b>	
	B	92	<b>18</b>	<b>18</b>	
	Totals	170	37	36 <sup>a</sup>	

Binomial Dispersion Test  $\chi^2 = 2.02$ , not significant

<sup>a</sup> Statistical significance  $p \leq 0.001$

\*Mitotic inhibition (%) =  $[1 - (\text{mean MI}_T / \text{mean MI}_C)] \times 100\%$   
 (where T = treatment and C = negative control)

Numbers highlighted exceed historical negative control range Appendix 5

**Table 2**  
**3 hour treatment +S-9, 17 hour recovery (3+17), Experiment 1**  
**Donor sex: male**

Treatment (µg/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	MIH* (%)
Vehicle	A	100	2	2	
	B	100	0	0	
	Totals	200	2	2	-
1582	A	100	0	0	
	B	100	0	0	
	Totals	200	0	0	0
2813	A	100	0	0	
	B	100	1	1	
	Totals	200	1	1	18
5000	A	100	0	0	
	B	100	0	0	
	Totals	200	0	0	11
CPA, 12.5	A	44	22	20	
	B	63	23	20	
	Totals	107	45	40 <sup>a</sup>	

Binomial Dispersion Test  $\chi^2 = 3.03$ , not significant

<sup>a</sup> Statistical significance  $p \leq 0.001$

\*Mitotic inhibition (%) =  $[1 - (\text{mean MI}_T / \text{mean MI}_C)] \times 100\%$

(where T = treatment and C = negative control)

Numbers highlighted exceed historical negative control range Appendix 5

**Table 3**  
**20 hour treatment -S-9, 0 hour recovery (20+0), Experiment 2**  
**Donor sex: male**

Treatment (µg/mL)	Replicate	Cells Scored	Cells with	Cells with	MIH* (%)
			Aberrations Including Gaps	Aberrations Excluding Gaps	
Vehicle	A	100	1	0	
	B	100	2	2	
	Totals	200	3	2	-
1311	A	100	5	3	
	B	100	1	1	
	Totals	200	6	4	0
2048	A	100	1	0	
	B	100	2	2	
	Totals	200	3	2	32
4000	A	100	3	3	
	B	100	0	0	
	Totals	200	3	3	60
NQO, 5.00	A	82	17	16	
	B	47	14	13	
	Totals	129	31	29 <sup>a</sup>	

Binomial Dispersion Test  $\chi^2 = 8.11$ , not significant

<sup>a</sup> Statistical significance  $p \leq 0.001$

\*Mitotic inhibition (%) =  $[1 - (\text{mean MI}_T / \text{mean MI}_C)] \times 100\%$

(where T = treatment and C = negative control)

Numbers highlighted exceed historical negative control range Appendix 5

**Table 4**  
**3 hour treatment +S-9, 17 hour recovery (3+17), Experiment 2**  
**Donor sex: male**

Treatment (µg/mL)	Replicate	Cells Scored	Cells with	Cells with	MIH* (%)
			Aberrations Including Gaps	Aberrations Excluding Gaps	
Vehicle	A	100	2	1	
	B	100	0	0	
	Totals	200	2	1	-
2048	A	100	2	2	
	B	100	0	0	
	Totals	200	2	2	2
3200	A	100	1	1	
	B	100	1	1	
	Totals	200	2	2	18
5000	A	100	5	4	
	B	100	2	2	
	Totals	200	7	6	25
CPA, 12.5	A	46	21	20	
	B	28	21	20	
	Totals	74	42	40 <sup>a</sup>	

Binomial Dispersion Test  $\chi^2 = 3.71$ , not significant

<sup>a</sup> Statistical significance  $p \leq 0.001$

\*Mitotic inhibition (%) =  $[1 - (\text{mean MI}_T / \text{mean MI}_C)] \times 100\%$

(where T = treatment and C = negative control)

Numbers highlighted exceed historical negative control range Appendix 5

## Appendix 2

### Abbreviations and classification of observations

abs = aberrations  
rep = replicate  
tot = total

#### Gaps (g)

csg = chromosome gap  
ctg = chromatid gap

#### Chromosome deletions (chr del)

del = chromosome deletion  
d min = double minute  
f = isolocus fragment

#### Chromosome exchanges (chr exch)

t = interchange between chromosomes (e.g. reciprocal translocation)  
inv = chromosome intrachange (e.g. pericentric inversion)  
dic = dicentric  
dic+f = dicentric with accompanying fragment  
acr = acentric ring  
r+f = centric ring with accompanying fragment  
r = centric ring

#### Chromatid deletions (ctd del)

del = chromatid deletion  
min = single minute

#### Chromatid exchanges (ctd exch)

qr = interchange between chromatids of different chromosomes (e.g. quadriradial)  
cx = obligate complex interchange  
e = chromatid intrachange  
tr/tr+f = isochromatid/chromatid interchange (triradial)/with accompanying fragment  
su = intra-arm intrachange with sister union of broken ends  
nud = intra-arm intrachange with non-union of broken ends distally  
nup = intra-arm intrachange with non-union of broken ends proximally

#### Other structural aberrations

pvz = pulverised cell  
mabs = multiple aberrations (greater than 7 aberrations per cell or too many aberrations to permit accurate analysis)

#### Numerical aberrations

E = endoreduplicated  
H = hyperdiploid (47-68 chromosomes)  
P = polyploid (greater than 68 chromosomes)

**Appendix 3**  
**Serine Endopeptidase (PPA 26797): summary of the numbers and types of structural aberrations observed**

**Table 5**  
**3 hour treatment -S-9, 17 hour recovery (3+17), Experiment 1**  
**Donor sex: male**

Treatment (µg/mL)	Rep	Cells *	G	Chr del	Chr exch	Ctd del	Ctd exch	Other	Abs +g	Abs -g
Vehicle	A	100	1	1	0	1	0	0	3	2
	B	100	1	0	0	0	0	0	1	0
	Total	200	2	1	0	1	0	0	4	2
1582	A	100	2	0	0	1	0	0	3	1
	B	100	0	0	0	1	0	0	1	1
	Total	200	2	0	0	2	0	0	4	2
2813	A	100	2	0	0	0	0	0	2	0
	B	100	0	0	0	0	0	0	0	0
	Total	200	2	0	0	0	0	0	2	0
5000	A	100	0	0	0	0	0	0	0	0
	B	100	1	0	0	0	0	0	1	0
	Total	200	1	0	0	0	0	0	1	0
NQO, 5.00	A	78	1	3	0	18	4	2	28	27
	B	92	3	3	0	21	5	1	33	30
	Total	170	4	6	0	39	9	3	61	57

\* Total cells examined for structural aberrations  
 Totals given for each culture may differ from values given in Appendix 1 if cells are observed which have more than one aberration  
 For abbreviations and classification see Appendix 2

**Table 6**  
**3 hour treatment +S-9, 17 hour recovery (3+17), Experiment 1**  
**Donor sex: male**

Treatment (µg/mL)	Rep	Cells *	G	Chr del	Chr exch	Ctd del	Ctd exch	Other	Abs +g	Abs -g
Vehicle	A	100	0	0	0	2	0	0	2	2
	B	100	0	0	0	0	0	0	0	0
	Total	200	0	0	0	2	0	0	2	2
1582	A	100	0	0	0	0	0	0	0	0
	B	100	0	0	0	0	0	0	0	0
	Total	200	0	0	0	0	0	0	0	0
2813	A	100	0	0	0	0	0	0	0	0
	B	100	0	0	0	1	0	0	1	1
	Total	200	0	0	0	1	0	0	1	1
5000	A	100	0	0	0	0	0	0	0	0
	B	100	0	0	0	0	0	0	0	0
	Total	200	0	0	0	0	0	0	0	0
CPA, 12.5	A	44	7	10	0	24	1	0	42	35
	B	63	5	6	0	27	1	0	39	34
	Total	107	12	16	0	51	2	0	81	69

\* Total cells examined for structural aberrations  
Totals given for each culture may differ from values given in Appendix 1 if cells are observed which have more than one aberration  
For abbreviations and classification see Appendix 2

**Table 7**  
**20 hour treatment -S-9, 0 hour recovery (20+0), Experiment 2**  
**Donor sex: male**

Treatment (µg/mL)	Rep	Cells *	G	Chr del	Chr exch	Ctd del	Ctd exch	Other	Abs +g	Abs -g
Vehicle	A	100	1	0	0	0	0	0	1	0
	B	100	0	0	0	2	0	0	2	2
	Total	200	1	0	0	2	0	0	3	2
1311	A	100	2	1	0	2	0	0	5	3
	B	100	0	0	0	1	0	0	1	1
	Total	200	2	1	0	3	0	0	6	4
2048	A	100	1	0	0	0	0	0	1	0
	B	100	0	0	0	2	0	0	2	2
	Total	200	1	0	0	2	0	0	3	2
4000	A	100	0	1	0	1	2	0	4	4
	B	100	0	0	0	0	0	0	0	0
	Total	200	0	1	0	1	2	0	4	4
NQO, 5.00	A	82	2	5	0	12	11	0	30	28
	B	47	5	1	0	12	14	1	33	28
	Total	129	7	6	0	24	25	1	63	56

\* Total cells examined for structural aberrations  
Totals given for each culture may differ from values given in Appendix 1 if cells are observed which have more than one aberration  
For abbreviations and classification see Appendix 2

**Table 8**  
**3 hour treatment +S-9, 17 hour recovery (3+17), Experiment 2**  
**Donor sex: male**

Treatment (µg/mL)	Rep	Cells *	G	Chr del	Chr exch	Ctd del	Ctd exch	Other	Abs +g	Abs -g
Vehicle	A	100	1	0	0	1	0	0	2	1
	B	100	0	0	0	0	0	0	0	0
	Total	200	1	0	0	1	0	0	2	1
2048	A	100	0	0	0	2	0	0	2	2
	B	100	0	0	0	0	0	0	0	0
	Total	200	0	0	0	2	0	0	2	2
3200	A	100	1	0	0	2	0	0	3	2
	B	100	0	0	0	1	0	0	1	1
	Total	200	1	0	0	3	0	0	4	3
5000	A	100	1	0	0	4	0	0	5	4
	B	100	1	0	0	2	0	0	3	2
	Total	200	2	0	0	6	0	0	8	6
CPA, 12.5	A	46	2	9	0	17	8	0	36	34
	B	28	1	10	0	25	4	0	40	39
	Total	74	3	19	0	42	12	0	76	73

\* Total cells examined for structural aberrations  
Totals given for each culture may differ from values given in Appendix 1 if cells are observed which have more than one aberration  
For abbreviations and classification see Appendix 2

**Appendix 4**  
**Serine Endopeptidase (PPA 26797): summary of the numbers and types of numerical aberrations observed**

**Table 9**  
**3 hour treatment -S-9, 17 hour recovery (3+17), Experiment 1**  
**Donor sex: male**

Treatment (µg/mL)	Rep	Cells **	H	E	P	Tot abs	% with num abs
Vehicle	A	101	0	1	0	1	1.0
	B	101	0	0	1	1	1.0
	Total	202	0	1	1	2	1.0
1582	A	100	0	0	0	0	0
	B	100	0	0	0	0	0
	Total	200	0	0	0	0	0
2813	A	100	0	0	0	0	0
	B	100	0	0	0	0	0
	Total	200	0	0	0	0	0
5000	A	101	1	0	0	1	1.0
	B	100	0	0	0	0	0
	Total	201	1	0	0	1	0.5
NQO, 5.00	A	78	0	0	0	0	0
	B	92	0	0	0	0	0
	Total	170	0	0	0	0	0

\*\* Total cells examined for numerical aberrations  
For abbreviations and classification see Appendix 2

**Table 10**  
**3 hour treatment +S-9, 17 hour recovery (3+17), Experiment 1**  
**Donor sex: male**

Treatment (µg/mL)	Rep	Cells **	H	E	P	Tot abs	% with num abs
Vehicle	A	101	0	0	1	1	1.0
	B	100	0	0	0	0	0
	Total	201	0	0	1	1	0.5
1582	A	100	0	0	0	0	0
	B	100	0	0	0	0	0
	Total	200	0	0	0	0	0
2813	A	100	0	0	0	0	0
	B	101	0	0	1	1	1.0
	Total	201	0	0	1	1	0.5
5000	A	100	0	0	0	0	0
	B	100	0	0	0	0	0
	Total	200	0	0	0	0	0
CPA, 12.5	A	44	0	0	0	0	0
	B	63	0	0	0	0	0
	Total	107	0	0	0	0	0

\*\* Total cells examined for numerical aberrations  
For abbreviations and classification see Appendix 2

**Table 11**  
**20 hour treatment -S-9, 0 hour recovery (20+0), Experiment 2**  
**Donor sex: male**

Treatment (µg/mL)	Rep	Cells **	H	E	P	Tot abs	% with num abs
Vehicle	A	100	0	0	0	0	0
	B	100	0	0	0	0	0
	Total	200	0	0	0	0	0
1311	A	100	0	0	0	0	0
	B	100	0	0	0	0	0
	Total	200	0	0	0	0	0
2048	A	101	0	0	1	1	1.0
	B	102	0	2	0	2	2.0
	Total	203	0	2	1	3	1.5
4000	A	102	0	0	2	2	2.0
	B	102	0	0	2	2	2.0
	Total	204	0	0	4	4	2.0
NQO, 5.00	A	82	0	0	0	0	0
	B	47	0	0	0	0	0
	Total	129	0	0	0	0	0

\*\* Total cells examined for numerical aberrations  
 Numbers highlighted exceed historical negative control range Appendix 5  
 For abbreviations and classification see Appendix 2

**Table 12**  
**3 hour treatment +S-9, 17 hour recovery (3+17), Experiment 2**  
**Donor sex: male**

Treatment (µg/mL)	Rep	Cells **	H	E	P	Tot abs	% with num abs
Vehicle	A	101	0	0	1	1	1.0
	B	100	0	0	0	0	0
	Total	201	0	0	1	1	0.5
2048	A	100	0	0	0	0	0
	B	100	0	0	0	0	0
	Total	200	0	0	0	0	0
3200	A	101	0	0	1	1	1.0
	B	100	0	0	0	0	0
	Total	201	0	0	1	1	0.5
5000	A	100	0	0	0	0	0
	B	101	0	0	1	1	1.0
	Total	201	0	0	1	1	0.5
CPA, 12.5	A	46	0	0	0	0	0
	B	28	0	0	0	0	0
	Total	74	0	0	0	0	0

\*\* Total cells examined for numerical aberrations

**Appendix 5**  
**Historical vehicle control ranges for human peripheral blood lymphocyte**  
**chromosome aberration (HLC) assay**

**Male**

		Structural aberrations observed on 100 cells scored		Numerical aberrations observed during scoring of structural aberrations	
		Structural aberrations including gaps	Structural aberrations excluding gaps	Polyploid cells	Numerical aberrations (H+E+P)
-S9	Number of studies	40	40	40	40
	Number of cultures	163	163	163	163
	Median	1	0	0	0
	Mean	1.04	0.72	0.26	0.37
	SD	1.37	1.01	0.49	0.61
	Observed range	0 – 8	0 – 5	0 – 2	0 – 3
	<b>95% reference range</b>	<b>0 – 5</b>	<b>0 – 3</b>	<b>0 – 1</b>	<b>0 – 2</b>
+S9	Number of studies	40	40	40	40
	Number of cultures	161	161	161	161
	Median	1	0	0	0
	Mean	1.04	0.67	0.30	0.44
	SD	1.11	0.86	0.58	0.73
	Observed range	0 – 5	0 – 4	0 – 3	0 – 3
	<b>95% reference range</b>	<b>0 – 4</b>	<b>0 – 3</b>	<b>0 – 2</b>	<b>0 – 2</b>

H = Hyperdiploid, E=Endoreduplicated, P = Polyploid

Reference ranges are calculated from percentiles of the observed distributions.

Calculated in February 2007 by CLEH Statistics, from audited report data of studies started between January 2005 and September 2006.

## Appendix 6 Quality control statements for S-9

### MOLTOX POST MITOCHONDRIAL SUPERNATANT (S-9) QUALITY CONTROL & PRODUCTION CERTIFICATE

<b>LOT NO.:</b> <u>2111</u>	<b>SPECIES:</b> <u>Rat</u>	<b>PREPARATION DATE:</b> <u>January 31, 2007</u>
<b>PART NO.:</b> <u>11-101</u>	<b>STRAIN:</b> <u>Sprague Dawley</u>	<b>EXPIRATION DATE:</b> <u>January 31, 2009</u>
<b>VOLUME:</b> <u>5ml</u>	<b>SEX:</b> <u>Male</u>	<b>BUFFER:</b> <u>0.154 M KCl</u>
	<b>TISSUE:</b> <u>Liver</u>	<b>INDUCING AGENT(s):</b> <u>Aroclor 1254</u>
<b>REFERENCE:</b> <u>Maron, D &amp; Ames, B. <i>Mutat Res</i> 113:173, 1983</u>		<u>(Monsanto KL615), 500 mg/kg i.p.</u>
<b>STORAGE:</b> <u>At or below -70°C</u>		

**BIOCHEMISTRY:**

- PROTEIN

35.3mg/ml

Assayed according to the method of Lowry et al., *JBC* 193:265, 1951 using bovine serum albumin as the standard.

- ALKOXYRESORUFIN-0-DEALKYLASE ACTIVITIES

<u>Activity</u>	<u>P450</u>	<u>Fold - Induction</u>
EROD	IA1, IA2	84.8
PROD	2B1	24.9
BROD	2B1	20.5
MROD	1A2	83.9

Assays for ethoxyresorufin-0-deethylase (EROD), pentoxy-, benzyl- and methoxyresorufin-0-dealkylases (PROD, BROD, & MROD) were conducted using a modification of the methods of Burke, et al., *Biochem Pharm* 34:3337, 1985. Fold-inductions were calculated as the ratio of the sample vs. uninduced specific activities (SA's). Control SA's (pmoles/min/mg protein) were 38.8, 16.5, 63.1, 8 12.3 for EROD, PROD, BROD and MROD, respectively.

**BIOASSAY:**

- TEST FOR THE PRESENCE OF ADVENTITIOUS AGENTS

Samples of S-9 were assayed for the presence of contaminating microflora by plating 1.0 ml volumes on Nutrient Agar and Minimal Glucose (Vogel-Bonner E, supplemented with 0.05 mM L-histidine and D-biotin) media. Triplicate plates were read after 40 - 48 h incubation at 35 ± 2°C. The tested samples met acceptance criteria.

- PROMUTAGEN ACTIVATION

No. His+ Revertants	
EtBr/ CPA/	
<u>TA98</u> <u>TA1535</u>	
107.6 1268	

The ability of the sample to activate ethidium (EtBr) and cyclophosphamide (CPA) to intermediates mutagenic to TA98 and TA1535, respectively, was determined according to Lesca, et al., *Mutation Res* 129:299, 1984. Data were expressed as revertants per µg EtBr or per mg CPA.

Dilutions of the sample S9, ranging from 0.2 - 10% in S9 mix, were tested for their ability to activate benzo(a)pyrene (BP) and 2-aminoanthracene (2-AA) to intermediates mutagenic to TA100. Assays were conducted using duplicate plates as described by Maron & Ames, (*Mutat Res* 113:173, 1983).

µl S9 per plate/number his<sup>-</sup> revertants per plate

<u>Promutagen</u>	<u>0</u>	<u>1</u>	<u>5</u>	<u>10</u>	<u>20</u>	<u>50</u>
BP (5 µg)	121	216	463	635	1126	1437
2-AA (2.5 µg)	104	1276	1784	1837	2235	1817

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**MOLTOX POST MITOCHONDRIAL SUPERNATANT (S-9)  
 QUALITY CONTROL & PRODUCTION CERTIFICATE**

**LOT NO.:** 2112                      **SPECIES:** Rat                      **PREPARATION DATE:** February 6, 2007  
**PART NO.:** 11-101                  **STRAIN:** Sprague Dawley              **EXPIRATION DATE:** February 6, 2009  
**VOLUME:** 5ml                      **SEX:** Male                      **BUFFER:** 0.154 M KCl  
   **TISSUE:** Liver                      **INDUCING AGENT(s):** Aroclor 1254  
**REFERENCE:** Maron, D & Ames, B. *Mutat Res* 113:173, 1983              (Monsanto KL615), 500 mg/kg i.p.  
**STORAGE:** At or below -70°C

**BIOCHEMISTRY:**

- PROTEIN

36.6mg/ml

Assayed according to the method of Lowry et al., *JBC* 193:265, 1951 using bovine serum albumin as the standard.

- ALKOXYRESORUFIN-0-DEALKYLASE ACTIVITIES

<u>Activity</u>	<u>P450</u>	<u>Fold - Induction</u>
EROD	1A1, 1A2	66.0
PROD	2B1	26.2
BROD	2B1	37.7
MROD	1A2	134.4

Assays for ethoxyresorufin-0-deethylase (EROD), pentoxy-, benzyl- and methoxyresorufin-0-dealkylases (PROD, BROD, & MROD) were conducted using a modification of the methods of Burke, et al., *Biochem Pharm* 34:3337, 1985. Fold-inductions were calculated as the ratio of the sample vs. uninduced specific activities (SA's). Control SA's (pmoles/min/mg protein) were 36.0, 15.6, 46.8, 8 9.8 for EROD, PROD, BROD and MROD, respectively.

**BIOASSAY:**

- TEST FOR THE PRESENCE OF ADVENTITIOUS AGENTS

Samples of S-9 were assayed for the presence of contaminating microflora by plating 1.0 ml volumes on Nutrient Agar and Minimal Glucose (Vogel-Bonner E, supplemented with 0.05 mM L-histidine and D-biotin) media. Triplicate plates were read after 40 - 48 h incubation at 35 ± 2°C. The tested samples met acceptance criteria.

- PROMUTAGEN ACTIVATION

No. His+ Revertants
EtBr/ CPA/ <u>TA98</u> <u>TA1535</u>
64.8      784

The ability of the sample to activate ethidium (EtBr) and cyclophosphamide (CPA) to intermediates mutagenic to TA98 and TA1535, respectively, was determined according to Lesca, et al., *Mutation Res* 129:299, 1984. Data were expressed as revertants per µg EtBr or per mg CPA.

Dilutions of the sample S9, ranging from 0.2 - 10% in S9 mix, were tested for their ability to activate benzo(a)pyrene (BP) and 2-aminoanthracene (2-AA) to intermediates mutagenic to TA100. Assays were conducted using duplicate plates as described by Maron & Ames, (*Mutat Res* 113:173, 1983).

µl S9 per plate/number his<sup>+</sup> revertants per plate

<u>Promutagen</u>	<u>0</u>	<u>1</u>	<u>5</u>	<u>10</u>	<u>20</u>	<u>50</u>
BP (5 µg)	105	347	612	752	844	790
2-AA (2.5 µg)	101	919	1213	1313	1136	710

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3-16-07

**Appendix 7**  
**Sponsor's certificate of analysis**



**Toxicology**

**Date: June 12, 2009**  
**Luna: 2007-20427-03**  
**Ref.: KM**

**Documentation of Test Material**  
Revised

**Product:** TOX BATCH  
**Batch:** PPA 26797  
**Type of enzyme:** Serine Endopeptidase  
**Host organism:** Bacillus licheniformis  
**Physical form / Colour:** Brownish liquid at room temperature  
**E.C.:** 3.4.21

**Activity:** 54600 PROT/g  
**Water (KF):** 88.1 % w/w  
**Dry matter:** 11.9 % w/w  
**Ash (600°C):** 2.4 % w/w  
**Total Organic Solids (TOS):** 9.5 % w/w  
**Specific gravity (g/ml):** 1.053 g/ml  
**pH:** 3.9  
**Total viable counts/g:** <200



**Study Director**



# TEST REPORT

**Serine endopeptidase (PPA 26797)**

**A 13-WEEK ORAL (GAVAGE) TOXICITY  
STUDY IN RATS**

**Amendment No 1 to Report**

<b>Study No:</b>	66063
<b>Sponsor Ref No:</b>	20076029
<b>Date:</b>	19 August 2009
<b>Author:</b>	████████████████████
<b>Number of pages:</b>	223
<b>Sponsor:</b>	Novozymes A/S Krogshøjvej 36 Safety & Toxicology DK-2880 Bagsværd Denmark

## Statement

This Amendment No 1 to report "Serine endopeptidase (PPA 26797) - A 13-Week Oral (Gavage) Toxicity Study in Rats" is prepared for the following reason:

- For confidentiality reasons, the internal Novozymes test item name, used in the development phase and toxicological studies of the enzyme, has been removed from the present study report and has been replaced by the generic enzyme name - Serine endopeptidase, followed by an unambiguous identification number of the test item batch number (PPA 26797). The sample identification number of the test item batch number, PPA 26797, is exactly the same as used in the original report.
- A new Principal Investigator report for Analysis of dose formulation has been issued in order to change the name to Serine endopeptidase (PPA 26797).
- [REDACTED] Enzyme Analytical Laboratory, Process Support Laboratories, Novozymes A/S, Krogshøjvej 36, DK-2880 Bagsværd, has been appointed new Principal Investigator instead of [REDACTED]

[REDACTED]

Study Director  
LAB Research (Scantox)

19 August 2009

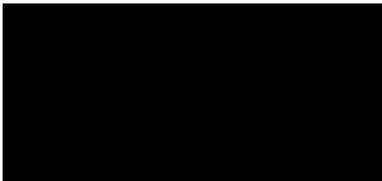
Date

## Good Laboratory Practice Compliance Statement

The study described in this Amendment No 1 to report "Serine endopeptidase (PPA 26797) - A 13-Week Oral (Gavage) Toxicity Study in Rats" was conducted under my supervision and responsibility and is in compliance with the OECD Principles of Good Laboratory Practice (as revised in 1997), which are in conformity with other international GLP regulations.

The report is a complete and accurate account of the methods employed and the data obtained.

Upon request of the Sponsor, this Amendment No 1 to report has been issued in order to change the test item name to Serine endopeptidase (PPA 26797).



Study Director  
LAB Research (Scantox)

19 August 2009

Date

## Quality Assurance Statement

Study number: 66063

Study title: Serine endopeptidase (PPA 26797) - A 13-Week Oral (Gavage) Toxicity Study in Rats

A review of the study plan has been performed and reported to the Study Director:

<b>Date of review:</b>	<b>Reporting date:</b>
15 May 2007	15 May 2007

The part of the study performed by LAB Research (Scantox) has been inspected by the Quality Assurance Unit at LAB Research (Scantox) in compliance with the principles of Good Laboratory Practice. Inspection reports have been communicated to the Study Director and to management on the dates stated in the table below. Process and facility inspections are performed on a regular basis in accordance with LAB Research (Scantox) procedures. Study-based inspection dates and the most recent inspection dates of the processes applicable to this study are stated in the below table.

Inspection type	Inspection item(s)	Inspection date(s)	Reporting date(s)
<b>Study-based</b>	Preparation of dose formulation	22 May 2007 08 August 2007	22 May 2007 08 August 2007
	Sampling of dose formulation	22 May 2007 13 August 2007	22 May 2007 13 August 2007
	Registration and storage of Test item	22 May 2007	22 May 2007
	Housing of animals	22 May 2007 09 July 2007	22 May 2007 09 July 2007
	Observation of animals	09 July 2007	09 July 2007
	Observation of animals, documentation	22 May 2007 09 August 2007	22 May 2007 09 August 2007
	Dosing	22 May 2007 09 July 2007	22 May 2007 09 July 2007
	Raw data	22 May 2007 09 August 2007 14 August 2007	22 May 2007 09 August 2007 14 August 2007
	Training records	08 August 2007 14 August 2007	08 August 2007 14 August 2007
	Open Field testing	09 August 2007	09 August 2007

	Stimuli-induced testing	14 August 2007	14 August 2007
	Blood sampling	20 August 2007	20 August 2007
	Necropsy	20 August 2007	20 August 2007
<b>Process-based</b>	Arrival and allocation of animals	03 May 2007	03 May 2007
	Re-allocation, weighing of animal and diet	08 May 2007 06 August 2007	08 May 2007 06 August 2007
	Sample dispatch	03 July 2007	03 July 2007
	Clinical chemistry analysis	05 July 2007	05 July 2007
	Haematology analysis	05 July 2007	05 July 2007
	Histology and pathology	24 January 2007 29 August 2007	24 January 2007 29 August 2007
	Ophthalmoscopy	19 March 2007 15 June 2007	19 March 2007 15 June 2007

The study report has been audited. As far as can be reasonably established, the methods, procedures and observations have been accurately described, and the results and data presented in the study report accurately reflect the raw data generated during the study.

The study report gives an accurate account of the methods and procedures outlined in the study plan and in LAB Research (Scantox) Standard Operating Procedures.

<b>Audit date(s) of Draft Report and data:</b>	<b>Reporting date (Study Director and management):</b>
18-20 September 2007, 24-28 September 2007 and 01 October 2007	01 October 2007
<b>Audit date of Final Report:</b>	
11 October 2007	
<b>Audit date of Amendment No 1 to report:</b>	<b>No report</b>
19 August 2009	

The part of the study performed by Novozymes A/S been inspected and the results reviewed by their Quality Assurance Unit and a test site QA statement has been issued.



19 August 2009

QA Auditor  
LAB Research (Scantox)

Date

## Personnel involved in the study

Study Director:

[REDACTED]

Study Supervisor:

[REDACTED]

Principal Investigator,  
Analysis of dose formulation:  
From study start until finalisation  
of Final Report:  
From June 2009 until finalisation  
of Amendment to Final Report:

[REDACTED]

[REDACTED]

Sponsor Monitor

[REDACTED]

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## 1 Summary

This study was conducted at LAB Research (Scantox), Hestehavevej 36A, Ejby, DK-4623 Lille Skensved, Denmark.

The objective of this study was to assess systemic toxicity of Serine endopeptidase (PPA 26797) in the rat when administered daily by oral gavage for a period of 13 weeks.

The present study was conducted in accordance with the methods recommended in the OECD Guideline No. 408, "Repeated Dose 90-day Oral Toxicity Study in Rodents", adopted 21 September 1998.

A total of 80 SPF Sprague Dawley rats (40 males and 40 females) was included in the study. The animals were allocated into four groups given vehicle (control; Group 1) and 50, 165.1 and 500.1 mg TOS/kg bw (Groups 2, 3 and 4, respectively). The test article was administered daily by oral gavage in a dose volume of 5 ml/kg. Clinical signs were recorded daily and the body weight and the food consumption were recorded weekly. Water consumption was recorded twice weekly. More extended clinical observations were performed weekly. Towards the end of the study, all animals were subjected to open field testing and stimuli-induced tests. Ophthalmoscopy was performed on all animals before start of treatment and on the animals in Groups 1 and 4 at the end of treatment. Blood samples for haematology and clinical chemistry were taken from all animals before end of treatment. At termination of the study, the animals were killed, examined macroscopically, weights of selected organs were recorded and selected tissues were evaluated microscopically.

No treatment related effects were seen at the clinical examinations, on the body weight, body weight gain, the food consumption, water consumption, on the clinical pathological parameters, at the ophthalmoscopic examination or on the organ weights.

At necropsy, no treatment related macroscopic changes were found. All microscopic findings reported were considered to be within the background incidence of findings reported in this age and strain of laboratory maintained rats and as such to be of no toxicological significance.

**In conclusion**, Serine endopeptidase (PPA 26797), given daily by oral gavage to rats for a period of 13 weeks at dosages of 50, 165.1 and 500.1 mg TOS/kg bw corresponding to 28747, 90090 and 287469 PROT/ kg bw, respectively (dose volume 5 ml/kg) caused no signs of toxicity. The no-observed-adverse-effect-level (NOAEL) was considered to be 500.1 mg TOS/kg body weight/day (corresponding to 287469 PROT/kg body weight/day).

## 2 Introduction

The objective of this study was to assess the toxicity of Serine endopeptidase (PPA 26797) administered daily by oral treatment to rats for 13 weeks.

The present study was performed in accordance with the OECD Guideline 408, adopted on 21 September 1998.

The rat was selected as the test model because of its proven suitability in this type of study. Oral treatment was chosen in order to comply with the possible human route of administration. The doses were selected by the Sponsor.

This study was conducted at LAB Research (Scantox), Hestehavevej 36A, Ejby, DK-4623 Lille Skensved, Denmark according to Study plan dated 15 May 2007 and Amendment No 1 dated 25 June 2007 and No 2 dated 09 July 2007.

The animals arrived on 15 May 2007. Treatment started on 22 May 2007. The in-life phase ended on 21 August 2007.

This report describes the procedures used and the results obtained.

## 3 Materials and methods

### 3.1 Test item and vehicle

The test item, Serine endopeptidase (PPA 26797) (batch No PPA 26797, expiry date 29 March 2017), was supplied by the Sponsor.

Test item characterisation (identity, purity, stability) was the responsibility of the Sponsor.

#### Test item:

Name: Serine endopeptidase (PPA 26797)  
Batch No: PPA 26797

#### Description of test item:

Activity: 54600 PROT/g  
Water (KF): 88.1% w/w

Dry matter:	11.9% w/w
Ash (600°C):	2.4% w/w
Total Organic Solids (TOS):	9.5% w/w
Specific gravity (g/ml):	1.053 g/ml
pH:	3.9
Total viable count:	<200

Physical form / Colour: Brownish liquid at room temperature

Storage condition: Deep frozen (-18°C)

Vehicle: Tap water. Analyses for relevant possible contaminants were performed regularly. Certificates of analysis are retained.

At request of the Sponsor, remaining test item was disposed of after completion of the treatment period.

### 3.2 Animals

The experiment was performed in 40 male and 40 female SPF Sprague Dawley rats of the Ntac:SD strain from Taconic Europe A/S, Ejby, Denmark. At the start of the acclimatisation period, the rats were approximately 5 weeks old and their body weight was within a range of +/- 20 grams for each sex. Ten (10) extra animals (5 of each sex) were available until completion of the acclimatisation period for replacement purposes.

An acclimatisation period of 7 days was allowed in order to reject animals in poor condition or at the extremes of the weight range.

### 3.3 Housing

The study took place in animal room No 116 provided with filtered air at a temperature of 21°C ±3°C and relative humidity of 55% ±15%. No deviations to these limits occurred. The temperature and relative humidity in the animal room were recorded hourly during the study and the records are retained.

The ventilation system has been designed to give 10 air changes per hour. The room was illuminated to give a cycle of 12 hours light and 12 hours darkness. Light was on from 06:00 h to 18:00 h.

The rats were kept in transparent polycarbonate cages (floor area: 1500 cm<sup>2</sup> - Height 21 cm) with two in each cage, males and females separated. The cages were cleaned and the bedding changed at least once per week.

Before the animals arrived, the animal room was cleaned and disinfected. During the study, the animal room was cleaned regularly and rinsed with water.

### **3.4 Bedding**

The bedding was softwood sawdust "Jeluxyl" from Jelu Werk GmbH, Josef Ehrler GmbH & Co KG, Ludwigsmühle, D-73494 Rosenberg, Germany. Analyses for relevant possible contaminants were performed regularly. Certificates of analysis are retained.

### **3.5 Environmental enrichment**

For environmental enrichment, the animals were offered a supply of Aspen Wood Wool from Tapvei Oy, FIN-73620 Kortteinen, Finland, at each change of bedding. Analyses for relevant possible contaminants were performed regularly. Certificates of analysis are retained.

Furthermore, an autoclaved brick of wood from Tapvei Oy, FIN-73620 Kortteinen, Finland, was provided to each cage. Analyses for relevant possible contaminants were performed regularly. Certificates of analysis are retained.

Each cage also contained a red transparent Rat House (Noryl, Tecniplast) from Tecniplast Gazzada S.a.r.l., 21020 Buguggiate -Va, Italy. The house allowed the animals to show a wide range of natural behaviour.

### **3.6 Diet**

A complete pelleted rodent diet "Altromin 1314 Fortified" (for growing animals) was available *ad libitum* until Day 49 of the dosing period. On Day 49 and throughout the study, the animals were offered *ad libitum* "Altromin 1324 Fortified" (for adult animals). Change of diet was performed one day earlier as compared to the study plan. However, this deviation

was considered not to have any effects on the outcome of the study. Altromin was supplied by Altromin Gesellschaft für Tierernährung mbH, D-32791 Lage, Germany. Analyses for major nutritive components and relevant possible contaminants were performed regularly. Certificates of analysis are retained.

### **3.7 Drinking water**

The animals had free access to bottles with domestic quality drinking water acidified with hydrochloric acid to pH 2.5 in order to prevent microbial growth. Analyses for relevant possible contaminants were performed regularly on the drinking water prior to acidification. Certificates of analysis are retained.

### **3.8 Animal randomisation and allocation**

On the day of arrival, the animals were allocated randomly to 4 groups and a group of extra animals, using a randomisation scheme.

Prior to commencement of treatment, the animals were re-allocated in order to reduce possible inter-group mean body weight differences. Data available from pre-treatment observations, clinical signs and laboratory investigations were taken into account when re-allocating animals.

On Day 3 of the study, the extra animals were killed.

### **3.9 Animal and cage identification**

Each animal was identified by punched earmarks.

Each cage was identified by a colour coded card containing at least study number, group number, sex and animal number.

### 3.10 Treatment

The groups, dose levels, animal numbers and colour codes were as follows:

Group	Dose*	Dose concentration*	Animal Nos		Colour code
	(v/v)	(mg TOS/kg bw)	Male	Female	
1	0%	0	1 - 10	11 - 20	White
2	10%	50	21 - 30	31 - 40	Blue
3	33%	165.1	41 - 50	51 - 60	Green
4	100%	500.1	61 - 70	71 - 80	Red

\*Material as supplied. (TOS, mg/kg = ml Test Item/kg bw x specific gravity x TOS, %w/w)

Example Group 2: 0.5 ml/kg bw x 1.053 g/ml x 9.5% w/w / 100% = 0.0500 g/kg bw= 50 mg/kg

- The daily dose was given by oral gavage according to the most recent body weight data.
- Treatment was performed daily for at least 91 days and until the day before necropsy.
- Dose volume was 5 ml/kg body weight.
- Dose formulations for Groups 2 to 4 were kept on a magnetic stirrer during treatment.
- Treatment was completed within twenty four (24) hours after preparation of the dose formulations.
- The first day of treatment was designated Day 1.

### 3.11 Dose formulation preparation

The dose formulation was prepared daily by diluting the test item in tap water. Treatment was completed within twenty four hours after the preparation of the dose formulations.

The test item was kept frozen at approximately  $-18^{\circ}\text{C}$  until use. Before use, each bottle of the test item was thawed in order to divide the contents into portions suitable for daily preparation of dose formulations and frozen again. The test item (original bottles or portions) was thawed overnight in the refrigerator or at room temperature for a maximum of 24 hours. Before dividing the contents of the original bottles into portions, the test item was stirred gently for at least 10 minutes on a magnetic stirrer.

Dose formulations were prepared as follows:

Group 1: Vehicle (Tap water)

Group 2: 1 portion of test item diluted in 9 portions of vehicle

Group 3: 1 portion of test item diluted in 2.03 portions of vehicle.

Group 4: Undiluted test item.

### **3.12 Control of dose preparations and usage**

Before preparation of dose formulation, the dose calculations were double checked.

Each step of the dose formulation preparation and the dosing, including weight of each dose formulation before and after dosing, was documented by weighing.

After dosing, the amount of dose formulation used for each group was compared with the predicted daily usage.

### **3.13 Analysis of dose formulations**

During week 1, 6 and 13, two (2) sets of triplicate (3) samples (6 samples in total) (each of 10 ml Cryotube, Nunc) of the four dose formulations were taken and stored frozen at approximately  $-18^{\circ}\text{C}$ . One set of triplicate samples is stored at LAB Research (Scantox) until the study is finalised and reported and can hereafter be discarded, if nothing else is agreed on. One set of triplicate sample was sent to the Sponsor for analysis.

The results of the analysis will be included in the final study report as [Appendix II](#)

### **3.14 Clinical signs**

#### **3.14.1 Daily observations**

All visible signs of ill health and any behavioural changes were recorded daily. Any deviation from normal was recorded with respect to time of onset, duration and intensity.

#### **3.14.2 Weekly observations**

Beginning prior to start of treatment, detailed clinical observations were performed outside the home cage once per week at similar times. Signs to be recorded included, but were not limited to: changes in skin/fur, eyes, mucous membranes, occurrence of secretions and

excretions and autonomic activity (*e.g.*, lacrimation, piloerection, pupil size, and unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypies (*e.g.* excessive grooming, repetitive circling) or bizarre behaviour (*e.g.* self-mutilation, walking backwards) were also recorded.

### **3.14.3 Open field and stimuli-induced tests**

On one occasion during the last two weeks of the study, all animals were examined with respect to reactivity to different types of stimuli (*e.g.* auditory, visual, tactile), grip strength and motor activity (open field test).

### **3.15 Body weight**

Starting at arrival, the animals were weighed once weekly, including Day -1 which was the body weight used for randomisation. During the dosing period, the animals were weighed on the last day of each study week (Days 7, 14, etc) and this weight was used for calculation of the doses for the following study weeks. Moreover, the animals were weighed on Day 1 and at necropsy.

### **3.16 Food consumption**

From Day 1, the food consumption was recorded weekly (Days 1, 7, 14 etc.) for each cage with a 7-day interval.

### **3.17 Water consumption**

From Day 1, the water consumption was recorded twice weekly for each cage.

### **3.18 Ophthalmoscopy**

Before start of treatment, ophthalmoscopy was performed on all animals. Before termination of treatment, all animals in Groups 1 and 4 were re-examined.

After application of tropicamide 1% solution (Mydriacyl, Alcon Universal Ltd., USA), both eyes were examined with an indirect ophthalmoscope and a portable slit-lamp microscope.

### 3.19 Clinical pathology

Before termination of treatment, blood samples were taken from all animals, and they were drawn from the sublingual venous plexus.

For haematology, at least 300 µl EDTA stabilised blood was taken. From this sample, a smear was prepared and stained with May-Grünwald and Giemsa for possible later manual differential leucocyte count (at extra cost). In case it is later decided to read all the smears manually, the manual counts will override the results of the ABX Pentra 120.

For the coagulation tests, 500 µl citrate stabilised blood was taken.

Approximately 750 µl blood was taken for clinical chemistry in plain glass tubes for serum.

At least 0.2 ml serum was transferred to cryotubes, labelled properly and stored at approximately -18°C until dispatch with dry ice to the Sponsor for possible future analysis. This analysis will not be part of this study

At necropsy, a bone marrow smear was taken from the femur of all animals (See the table under the heading [Organs and tissues](#)). The smears were fixed and stained with May-Grünwald and Giemsa stain. These smears were not analysed as there were no indications for this analysis based on the haematological findings.

The parameters, methods and units for the laboratory investigations are stated below:

### 3.19.1 Haematology and coagulation parameters

Parameter	Method/Equipment	Unit
Haemoglobin (Hb)	Direct measurement/ABX Pentra DX120SPS	mmol/l
Red blood cell count (RBC)	Direct measurement/ABX Pentra DX120SPS	10 <sup>12</sup> /l
Haematocrit (HT)	Direct measurement/ABX Pentra DX120SPS	ml/100 ml
Mean cell volume (MCV)	Calculated/ABX Pentra DX120SPS	fl
Mean cell haemoglobin (MCH)	Calculated/ABX Pentra DX120SPS	fmol
Mean cell haemoglobin concentration (MCHC)	Calculated/ABX Pentra DX120SPS	mmol/l
White blood cell count (WBC)	Direct measurement/ABX Pentra DX120SPS	10 <sup>9</sup> /l
Differential leucocyte count (NEUTRO, LYMPHO, EOS, BASO, MONO)	Direct measurement/ABX Pentra DX120SPS	% and 10 <sup>9</sup> /l
Platelet count (Plt)	Direct measurement/ABX Pentra DX120SPS	10 <sup>9</sup> /l
Activated partial thromboplastin time (APTT)	IL Test <sup>TM</sup> /ACL <sup>TM</sup> (*)	sec.
Prothrombin time (Pt)	IL Test <sup>TM</sup> /ACL <sup>TM</sup> (*)	sec.
Fibrinogen (Fib)	IL Test <sup>TM</sup> /ACL <sup>TM</sup> (*)	g/l

(\* Instrumentation Laboratories, Automated Coagulation Laboratory)

### 3.19.2 Clinical chemistry

Parameter	Method	Unit
Alanine aminotransferase (ALAT)	Hitachi 917	μkat/l
Aspartate aminotransferase (ASAT)	Hitachi 917	μkat/l
Alkaline phosphatase (ALKPH)	Hitachi 917	μkat/l
Bilirubin (total) (BILI)	Hitachi 917	μmol/l
Gamma-glutamyl transferase (GGT)	Hitachi 917	μkat/l
Cholesterol (CHOL)	Hitachi 917	mmol/l
Triglycerides (TRIG)	Hitachi 917	mmol/l
Carbamide (UREA)	Hitachi 917	mmol/l
Creatinine (CREAT)	Hitachi 917	μmol/l
Glucose (GLUC)	Hitachi 917	mmol/l
Sodium (Na)	Ion selective electrode/Hitachi 917	mmol/l
Potassium (K)	Ion selective electrode/Hitachi 917	mmol/l
Calcium (Ca)	Hitachi 917	mmol/l
Magnesium (Mg)	Hitachi 917	mmol/l
Inorganic phosphorus (P)	Hitachi 917	mmol/l
Chloride (Cl)	Ion selective electrode/Hitachi 917	mmol/l
Protein (total) (PROTEIN)	Hitachi 917	g/l
Albumin (ALB)	Hitachi 917	g/l
Globulin	Calculated	g/l
Albumin/Globulin (ALB/G) ratio	Calculated	No unit

### 3.20 Terminal observations

On the day of necropsy, the animals were weighed, examined externally and placed in a chamber with atmospheric air upon which CO<sub>2</sub> is applied at a steadily increasing concentration for euthanasia. The animals were monitored closely while in the chamber. Death was confirmed and the animals were bled before proceeding. The animals were necropsied in the sequence of one or two animals/group.

### **3.20.1 Necropsy**

A macroscopic examination was performed after opening the cranial, thoracic and abdominal cavities and by observing the appearance of the organs and tissues *in situ*. Any macroscopic change was recorded with details of the location, colour, shape and size in the PathData computer system.

### **3.20.2 Organs and tissues**

Either whole organs or selected samples of the indicated organs and tissues were subjected to the procedures itemised in the list given below. Weights were recorded in the PathData computer system.

Paired organs were weighed together. The relative organ weights, i.e. the organ weight as a percentage of the body weight and organ weight as a percentage of the brain weight, were calculated for each animal.

All tissues were initially fixed in phosphate buffered neutral 4% formaldehyde with the exception of the eyes and testes (Modified Davidsons's fixative). The fixative for long term preservation was phosphate buffered neutral 4% formaldehyde for all tissues. The lungs were infused with fixative at necropsy.

Organs and tissues	W e i g h	F i x	M i c r o	Organs and tissues	W e i g h	F i x	M i c r o
Abnormalities (gross lesions)		x	x	Pituitary		x	x
Adrenals	x	x	x	Prostate		x	x
Aorta (thoracic)		x	x	Salivary glands (right parotid, sublingual and submandibular)		x	x
Brain	x	x	x	Sciatic nerve		x	x
Bone marrow smear		x		Seminal vesicles		x	x
Epididymides	x	x	x	Skeletal muscle		x	x
Eyes with lens/optic nerve		x	x	Skin		x	x
Heart	x	x	x	Spinal cord (cervical, thoracic, lumbar)		x	x
Intestine small (duodenum, jejunum, ileum)		x	x	Spleen	x	x	x
Intestine large (caecum, colon, rectum)		x	x	Sternum (for bone marrow)		x	x
Kidneys	x	x	x	Stomach (glandular, non glandular)		x	x
Liver	x	x	x	Testes	x	x	x
Lungs		x	x	Thymus	x	x	x
Lymph nodes (mesenteric and right mandibular)		x	x	Thyroids (incl. parathyroid)		x	x
Mammary gland		x	x	Trachea		x	x
Oesophagus		x	x	Urinary bladder		x	x
Ovaries	x	x	x	Uterus (horn, cervix and oviducts)	x	x	x
Pancreas		x	x	Vagina		x	x

### 3.20.3 Processing and microscopic examination

After fixation, the organs and tissues sampled for microscopic examination were trimmed and representative specimens were taken for histological processing. The specimens were embedded in paraffin and cut at a nominal thickness of approximately 5 µm, stained with haematoxylin and eosin and examined under a light microscope. Paired organs were processed together.

All pathological findings were entered directly onto the PathData computer system.

Histological alterations were graded on a 5-grade system:

- Grade 1 - Minimal/Very few/Very small
- Grade 2 - Slight/Few/Small
- Grade 3 - Moderate/Moderate number/Moderate size
- Grade 4 - Marked/Many/Large
- Grade 5 - Massive/Extensive number/Extensive size
- Present - Finding present/Severity not scored

The following organs and tissues were examined microscopically:

- All organs and tissues from all control (Group 1) and high dose animals (Group 4).
- From all animals, the organs and tissues where treatment-related changes were observed in the high dose group (at extra cost).
- All organs and tissues from all animals dead after initiation of treatment.
- All gross lesions from all animals.

Submandibular lymph nodes with macroscopic visible signs of accumulation of blood due to blood sampling from the sublingual plexus were fixed but not processed histologically.

Tissues not examined microscopically were stored at LAB Research (Scantox) held in fixative.

#### **3.20.4 Peer review**

A peer review by a LAB Research (Scantox) peer reviewing pathologist was performed on selected slides. Diagnostic discrepancies were resolved by discussion.

#### **3.21 Statistics**

Data was processed to give group mean values and standard deviations where appropriate.

Thereafter each continuous variable was tested for homogeneity of variance with Levene's test. If the variance was homogeneous, analysis of variance was carried out for the variable. If any significant differences were detected, possible inter-group differences were assessed with Dunnett's test (comparing treated groups with a control group). If the variance was heterogeneous, each variable was tested for normality by the Shapiro-Wilk method. In case of normal distribution, possible inter-group differences were identified with Student's t-test.

Otherwise the possible inter-group differences were assessed by Kruskal-Wallis's test. If any significant inter-group differences were detected, the subsequent identification of the groups was carried out with Wilcoxon Rank-Sum test.

For all tests, the level of significance was defined as  $p < 0.05$ .

The statistical analyses were made with SAS<sup>®</sup> procedures (version 8.2) described in "SAS/STAT<sup>®</sup> User's Guide, SAS OnlineDoc<sup>®</sup>, 1999, SAS Institute Inc., Cary, North Carolina 27513, USA.

## **3.22 Archives**

### **3.22.1 LAB Research (Scantox)**

For a period of 10 years, LAB Research (Scantox) will be responsible for the archiving of the following materials relating to the study:

Study plan, study plan amendments and correspondence, test material receipts, sample of test item, animal records, all original data, wet tissues, blocks and slides and final report.

At the end of the storage period, LAB Research (Scantox) will contact the Sponsor for instructions whether the material should be transferred, retained or destroyed. Implementation of such instructions will be at additional costs to the Sponsor.

### **3.22.2 Novozymes A/S (Analysis of dose formulation)**

For a period of 10 years, the raw data pertaining to formulation analysis, shipping documents, correspondence and the analytical report will be archived at Novozymes A/S.

## 4 Results

### 4.1 Mortality

No treatment related deaths occurred.

Animal Nos 21 (Dose Group No 2, 50 mg TOS/kg/day), 42 (Dose group No 3, 165.1 mg TOS/kg/day) and 51 (Dose group No 3, 165.1 mg TOS/kg/day) were found dead on Days 33, 25 and 7 of the study, respectively. Animal No 55 (Dose group No 3, 165.1 mg TOS/kg/day) was sacrificed moribund on Day 8 of the study.

The changes observed at necropsy of these animals were all considered to be the result of gavage accidents.

### 4.2 Clinical signs

[Table 1](#) [Table 2](#) [Table 9](#) [Table 10](#) [Table 11](#)

No treatment related clinical signs were observed at the daily check or at the detailed weekly clinical observations.

No treatment related changes were seen with respect to stimuli-induced sensory reactivity and open field behaviour.

The increased number of rearings observed for the males in Group 3 was considered incidental due to the occurrence in one sex only and due to the lack of dose dependency.

### 4.3 Body weight

[Table 3](#) [Table 12](#)

No treatment related effects were seen on the body weights.

### 4.4 Food consumption

[Table 4](#) [Table 13](#)

No treatment related effects were seen on the food consumption.

The significantly higher food consumption observed for the males in Group 3 on Days 14 and 21 was considered incidental due to the occurrence on these days only, due to the occurrence in one sex only and due to the lack of dose dependency.

#### **4.5 Water consumption**

##### [Table 5 Table 14](#)

On some occasions during the study, a statistically significantly increased water consumption was seen for the animals in Group 4. On a few occasions, this was also the case for the males in Groups 2 and 3 and for the females in Group 3.

Due to the absence of any other adverse findings in all parameters evaluated, including the histopathological examination, this was not considered an adverse effect of the test item per se, but linked to the palatability of the test item, increasing the water consumption.

#### **4.6 Ophthalmoscopy**

##### [Table 15](#)

No treatment related changes were seen at the ophthalmoscopic examinations.

#### **4.7 Haematology**

##### [Table 6 Table 16](#)

No treatment related effects were seen on the haematological parameters.

The lower white blood cell count observed for the females in Group 3 was considered incidental as it occurred in one sex only and as no dose dependent effects were seen.

#### **4.8 Clinical chemistry**

##### [Table 7 Table 17](#)

No treatment related effects were seen on the clinical chemical parameters.

The activity of alkaline phosphatase was higher for the males in Group 4. In addition, the activity of alanine aminotransferase was lower for the females in Groups 2-4 and the activity of asparagine aminotransferase was lower for the females in Group 4. As the above mentioned findings were present in one sex only and as no clear dose dependency was seen, these findings were considered incidental. This was supported by the lack of any adverse findings at the histopathological examination.

#### **4.9 Organ weights**

##### [Table 8 Table 18](#)

No treatment related effects were seen on the organ weights.

The relative weight of the liver was lower for the males in Group 3 as compared with the control group. This was considered incidental for the same reasons stated above.

#### **4.10 Macroscopic findings**

##### [Appendix I](#)

All changes reported at necropsy of the scheduled deaths were considered incidental.

##### Decedent animals

Animal No 21 (Dose group No 2, 50 mg TOS/kg/day) was found dead on Day 33 of the study. At necropsy, a reddish fluid was found in the thoracic cavity.

Animal No 42 (Dose group No 3, 165.1 mg TOS/kg/day) was found dead on Day 25 of the study. Oedema was recorded in the abdominal cavity and in the thoracic cavity. Likewise a reddish fluid was present in the thoracic cavity.

Animal No 51 (Dose group No 3, 165.1 mg TOS/kg/day) was found dead on Day 7 of the study. Blood clots were observed in the thoracic cavity. In addition to this, a red discolouration of the lungs and thymus was recorded.

Animal No 55 (Dose group No 3, 165.1 mg TOS/kg/day) was sacrificed moribund on Day 8 of the study. Blood clots were observed in the thoracic cavity. In addition to this, a red discolouration of the lungs and perforation of the oesophagus were found.

The changes observed at necropsy of the decedent animals in this study were all considered to be the result of gavage accidents.

#### **4.11 Microscopic findings**

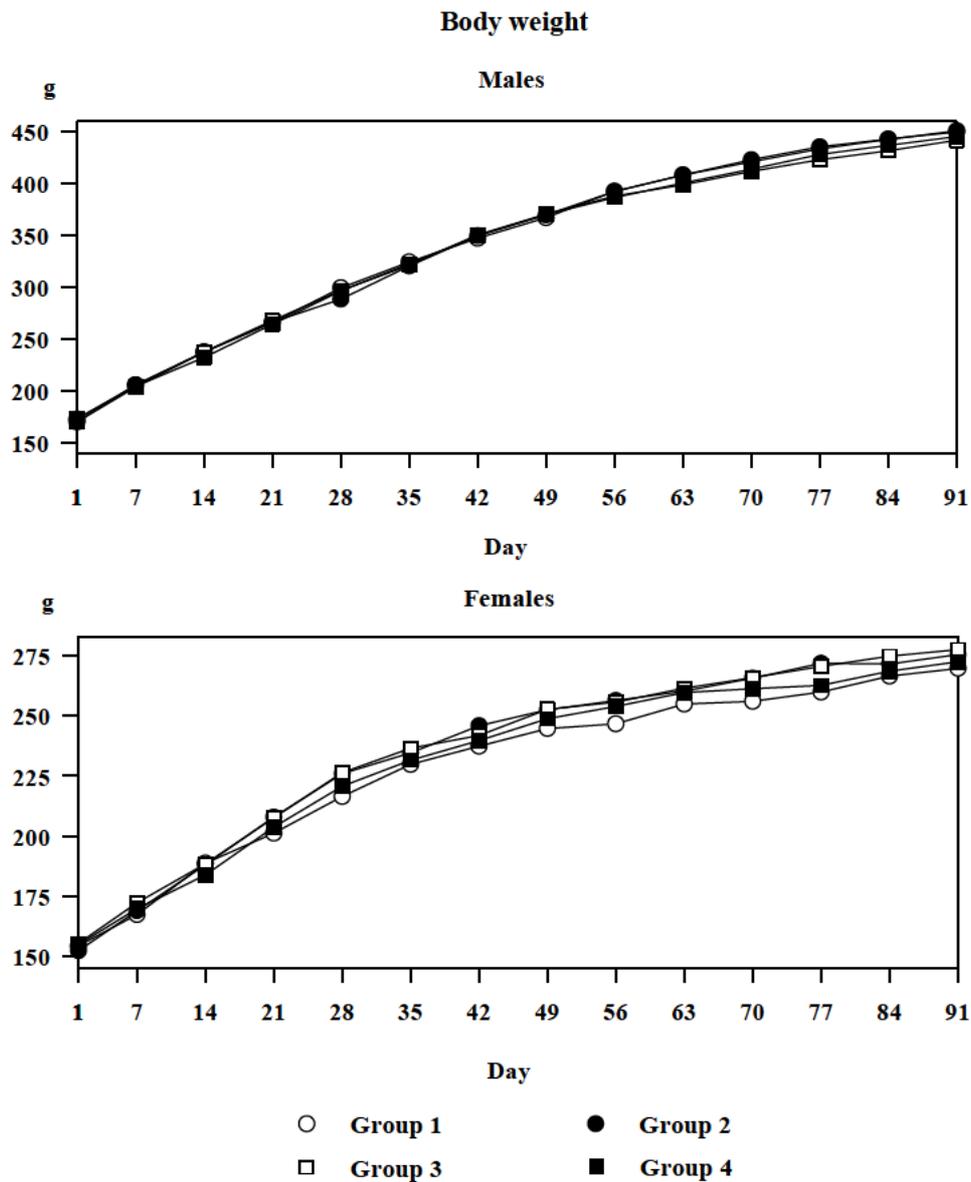
##### [Appendix I](#)

All microscopic findings reported were considered to be within the background incidence of findings reported in this age and strain of laboratory maintained rats and as such to be of no toxicological significance.

#### **Conclusion**

Serine endopeptidase (PPA 26797), given daily by oral gavage to rats for a period of 13 weeks at dosages of 50, 165.1 and 500.1 mg TOS/kg bw corresponding to 28747, 90090 and 287469 PROT/ kg bw, respectively (dose volume 5 ml/kg) caused no signs of toxicity. The no-observed-adverse-effect-level (NOAEL) was considered to be 500.1 mg TOS/kg body weight/day (corresponding to 287469 PROT/kg body weight/day).

**Figure 1 Body weight**



**Table 1 Open field testing – Group mean values**

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Open field testing

Group mean values

Males

GROUP	TIME MOVING				TOTAL DISTANCE (m)				NO. OF REARINGS				TIME CENTRE			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	239.4	14.5	10		37.1	6.9	10		32.0	7.3	10		27.2	23.6	10	
2	240.8	10.6	9		39.3	5.2	9		29.0	7.5	9		21.8	7.2	9	
3	242.1	8.1	9		39.8	6.4	9		45.0	17.5	9	*	17.8	10.1	9	
4	242.6	13.1	10		39.1	5.9	10		33.7	8.1	10		19.3	6.6	10	

GROUP	TIME PERIPHERY				TOTAL CORNER VISITS				MOVES/COUNTS				FAECES			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	272.8	23.6	10		21.0	5.8	10		1197.6	72.3	10		1.7	1.9	10	
2	278.2	7.2	9		22.8	5.8	9		1203.3	53.6	9		0.8	1.6	9	
3	282.2	10.1	9		21.2	4.8	9		1210.1	41.1	9		0.6	1.1	9	
4	280.7	6.6	10		21.0	4.5	10		1213.6	65.3	10		1.8	1.9	10	

\* means 0.01<p<0.05, versus control group

S.D. = standard deviation N = numbers of cages

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Open field testing

Group mean values

Females

GROUP	TIME MOVING				TOTAL DISTANCE (m)				NO. OF REARINGS				TIME CENTRE			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	235.1	30.8	10		46.8	10.8	10		43.5	20.9	10		9.2	4.3	10	
2	244.1	8.8	10		46.7	7.2	10		39.3	12.3	10		6.0	2.7	10	
3	240.6	6.3	8		45.1	5.4	8		39.3	9.9	8		5.3	3.4	8	
4	224.8	61.8	10		42.1	13.7	10		38.0	14.5	10		6.6	5.4	10	

GROUP	TIME PERIPHERY				TOTAL CORNER VISITS				MOVES/COUNTS				FAECES			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	290.8	4.3	10		27.2	8.8	10		1175.1	154.9	10		0.4	1.3	10	
2	294.0	2.7	10		29.2	9.5	10		1220.5	43.9	10		0.0	0.0	10	
3	294.8	3.4	8		26.6	3.9	8		1202.6	30.5	8		0.0	0.0	8	
4	293.4	5.4	10		23.9	8.1	10		1124.0	308.9	10		0.5	1.3	10	

p>0.05, versus control group

S.D. = standard deviation N = numbers of cages

**Table 2 Stimuli-induced clinical observations – Incidence of findings**

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Stimuli-induced clinical observations

Incidence of findings

Males

GROUP	PUPIL REFLEX		Total	p
	Proper reaction	Failed reaction		
1	10	0	10	
2	9	0	9	
3	8	1	9	
4	10	0	10	
Total	37	1	38	

GROUP	TOE PINCH REACTION	Total	p
	Proper reaction		
1	10	10	
2	9	9	
3	9	9	
4	10	10	
Total	38	38	

p>0.05 versus control group

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Stimuli-induced clinical observations

Incidence of findings

Males

GROUP	GRASP RESPONSE		Total	p
	Proper reaction	Failed reaction		
1	10	0	10	
2	9	0	9	
3	8	1	9	
4	9	1	10	
Total	36	2	38	

GROUP	GRIP STRENGTH	Total	p
	Proper reaction		
1	10	10	
2	9	9	
3	9	9	
4	10	10	
Total	38	38	

p>0.05 versus control group

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Stimuli-induced clinical observations

Incidence of findings

Males

GROUP	EYELID REFLEX	Total	p
	Proper reaction		
1	10	10	
2	9	9	
3	9	9	
4	10	10	
Total	38	38	

GROUP	STARTLE RESPONSE	Total	p
	Proper reaction		
1	10	10	
2	9	9	
3	9	9	
4	10	10	
Total	38	38	

p>0.05 versus control group

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Stimuli-induced clinical observations

Incidence of findings

Males

GROUP	HEAD SHAKE RESPONSE	Total	p
	Proper reaction		
1	10	10	
2	9	9	
3	9	9	
4	10	10	
Total	38	38	

GROUP	RIGHTING REFLEX, TABLE	Total	p
	Proper reaction		
1	10	10	
2	9	9	
3	9	9	
4	10	10	
Total	38	38	

p>0.05 versus control group

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Stimuli-induced clinical observations

Incidence of findings

Males

GROUP	RIGHTING REFLEX, HAND	Total	p
	Proper reaction		
1	10	10	
2	9	9	
3	9	9	
4	10	10	
Total	38	38	

GROUP	PLACING REFLEX	Total	p
	Proper reaction		
1	10	10	
2	9	9	
3	9	9	
4	10	10	
Total	38	38	

p>0.05 versus control group

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Stimuli-induced clinical observations

Incidence of findings

Males

GROUP	NEGATIVE GEOTAXIS		Total	p
	Proper reaction	Failed reaction		
1	9	1	10	
2	9	0	9	
3	9	0	9	
4	8	2	10	
Total	35	3	38	

p>0.05 versus control group

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Stimuli-induced clinical observations

Incidence of findings

Females

GROUP	PUPIL REFLEX	Total	p
	Proper reaction		
1	10	10	
2	10	10	
3	7	7	
4	10	10	
Total	37	37	

GROUP	TOE PINCH REACTION	Total	p
	Proper reaction		
1	10	10	
2	10	10	
3	7	7	
4	10	10	
Total	37	37	

p>0.05 versus control group

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Stimuli-induced clinical observations

Incidence of findings

Females

GROUP	GRASP RESPONSE		Total	p
	Proper reaction	Failed reaction		
1	10	0	10	
2	10	0	10	
3	7	0	7	
4	9	1	10	
Total	36	1	37	

GROUP	GRIP STRENGTH	Total	p
	Proper reaction		
1	10	10	
2	10	10	
3	7	7	
4	10	10	
Total	37	37	

p>0.05 versus control group

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Stimuli-induced clinical observations

Incidence of findings

Females

GROUP	EYELID REFLEX	Total	p
	Proper reaction		
1	10	10	
2	10	10	
3	7	7	
4	10	10	
Total	37	37	

GROUP	STARTLE RESPONSE	Total	p
	Proper reaction		
1	10	10	
2	10	10	
3	7	7	
4	10	10	
Total	37	37	

p>0.05 versus control group

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Stimuli-induced clinical observations

Incidence of findings

Females

GROUP	HEAD SHAKE RESPONSE	Total	p
	Proper reaction		
1	10	10	
2	10	10	
3	7	7	
4	10	10	
Total	37	37	

GROUP	RIGHTING REFLEX, TABLE	Total	p
	Proper reaction		
1	10	10	
2	10	10	
3	7	7	
4	10	10	
Total	37	37	

p>0.05 versus control group

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Stimuli-induced clinical observations

Incidence of findings

Females

GROUP	RIGHTING REFLEX, HAND	Total	p
	Proper reaction		
1	10	10	
2	10	10	
3	7	7	
4	10	10	
Total	37	37	

GROUP	PLACING REFLEX	Total	p
	Proper reaction		
1	10	10	
2	10	10	
3	7	7	
4	10	10	
Total	37	37	

p>0.05 versus control group

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Stimuli-induced clinical observations

Incidence of findings

Females

GROUP	NEGATIVE GEOTAXIS		Total	p
	Proper reaction	Failed reaction		
1	9	1	10	
2	10	0	10	
3	7	0	7	
4	10	0	10	
Total	36	1	37	

p>0.05 versus control group

**Table 3 Body weight – Group mean values**

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Body weight and body weight gain (g)

Group mean values - From arrival to Day 91

Males

GROUP	ON ARRIVAL				DAY OF RE-ALLOCATION				DAY 1				DAY 7				DAY 14			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	132.6	6.6	10		168.0	9.2	10		172.4	10.0	10		205.3	10.9	10		237.5	14.7	10	
2	132.2	6.1	10		167.5	8.0	10		169.7	13.9	10		205.7	10.7	10		237.2	10.5	10	
3	130.2	5.3	10		167.8	5.4	10		169.9	8.4	10		203.5	9.2	10		237.5	11.0	10	
4	134.4	6.1	10		168.3	7.5	10		173.0	6.6	10		204.3	9.5	10		232.0	10.2	10	

GROUP	DAY 21				DAY 28				DAY 35				DAY 42				DAY 49			
	Mean	S.D.	N	p																
1	265.7	17.0	10		299.2	20.0	10		324.2	22.3	10		347.2	23.9	10		367.1	21.2	10	
2	266.3	10.8	10		288.3	20.8	10		320.3	14.6	9		349.7	16.0	9		369.6	19.4	9	
3	267.7	12.3	10		296.8	14.1	9		321.1	15.0	9		350.3	14.1	9		370.6	15.3	9	
4	264.1	7.5	10		296.3	10.3	10		322.4	11.8	10		349.6	13.7	10		370.2	13.1	10	

p>0.05, versus control group

S.D. = standard deviation N = number of animals

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Body weight and body weight gain (g)

Group mean values - From arrival to Day 91

Males

GROUP	DAY 56				DAY 63				DAY 70				DAY 77				DAY 84			
	Mean	S.D.	N	p																
1	392.5	20.9	10		408.2	24.1	10		421.1	26.2	10		433.5	30.8	10		442.8	33.1	10	
2	392.3	21.5	9		408.2	23.0	9		423.0	22.3	9		435.6	25.9	9		442.9	25.1	9	
3	387.8	15.3	9		398.8	15.3	9		411.8	17.6	9		423.0	18.9	9		431.7	21.2	9	
4	386.4	15.6	10		400.5	17.6	10		414.0	18.8	10		428.1	19.8	10		436.9	21.6	10	

GROUP	DAY 91				BODY WT GAIN DAY 1 TO 91			
	Mean	S.D.	N	p	Mean	S.D.	N	p
1	450.9	37.2	10		278.5	32.5	10	
2	449.9	27.2	9		281.4	20.9	9	
3	441.8	21.9	9		271.7	28.1	9	
4	445.3	23.0	10		272.3	26.0	10	

p>0.05, versus control group

S.D. = standard deviation N = number of animals

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Body weight and body weight gain (g)

Group mean values - From arrival to Day 91

Females

GROUP	ON ARRIVAL				DAY OF RE-ALLOCATION				DAY 1				DAY 7				DAY 14			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	127.6	7.9	10		151.3	7.3	10		154.3	7.5	10		167.3	7.9	10		188.7	11.0	10	
2	126.4	7.2	10		151.3	10.1	10		152.2	10.2	10		168.9	8.0	10		188.0	9.8	10	
3	126.5	11.1	10		151.6	10.2	10		155.2	10.9	10		172.2	10.1	10		188.4	13.0	8	
4	127.3	7.7	10		151.2	6.0	10		154.8	6.2	10		169.7	7.8	10		183.7	10.3	10	

GROUP	DAY 21				DAY 28				DAY 35				DAY 42				DAY 49			
	Mean	S.D.	N	p																
1	201.2	15.0	10		216.4	10.9	10		229.8	13.9	10		237.4	16.2	10		244.7	17.8	10	
2	207.8	16.4	10		225.9	11.3	10		234.6	7.8	10		245.9	10.0	10		252.5	11.0	10	
3	207.6	15.3	8		226.4	14.3	8		236.5	11.5	8		241.9	14.8	8		252.8	13.8	8	
4	203.7	9.8	10		220.7	10.8	10		231.5	14.3	10		239.7	14.6	10		248.8	11.7	10	

p>0.05, versus control group

S.D. = standard deviation N = number of animals

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Body weight and body weight gain (g)

Group mean values - From arrival to Day 91

Females

GROUP	DAY 56				DAY 63				DAY 70				DAY 77				DAY 84			
	Mean	S.D.	N	p																
1	246.7	13.4	10		254.9	10.9	10		256.1	11.7	10		259.9	13.6	10		266.5	12.0	10	
2	256.4	9.5	10		260.3	10.0	10		265.7	9.4	10		271.9	13.5	10		271.6	10.3	10	
3	255.8	10.1	8		261.5	12.6	8		265.9	19.0	8		270.6	15.7	8		274.9	12.4	8	
4	253.9	15.5	10		259.7	16.6	10		261.3	14.5	10		262.7	12.8	10		268.6	17.7	10	

GROUP	DAY 91				BODY WT GAIN DAY 1 TO 91			
	Mean	S.D.	N	p	Mean	S.D.	N	p
1	269.8	13.0	10		115.5	11.3	10	
2	275.5	10.1	10		123.3	13.1	10	
3	277.6	11.2	8		122.4	8.3	8	
4	272.5	17.9	10		117.7	15.9	10	

p>0.05, versus control group

S.D. = standard deviation N = number of animals

**Table 4 Food consumption – Group mean values**

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Food consumption (g)

Group mean values per animal - Day 1 - Day 91

Males

GROUP	DAY 7				DAY 14				DAY 21				DAY 28				DAY 35			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	157.1	6.6	5		180.6	7.0	5		182.0	8.2	4		180.3	7.4	5		171.4	9.8	5	
2	164.4	6.4	5		185.6	4.8	5		193.1	9.2	5		178.8	27.1	5		181.4	15.6	4	
3	161.3	12.6	5		199.6	7.7	5	**	203.9	11.5	5	*	187.1	12.0	4		181.5	8.5	5	
4	160.8	21.4	5		190.3	12.8	5		194.1	11.7	5		188.4	10.4	5		184.9	11.2	5	

GROUP	DAY 42				DAY 49				DAY 56				DAY 63				DAY 70			
	Mean	S.D.	N	p																
1	162.5	9.1	4		174.5	7.9	5		169.2	6.8	5		181.0	9.7	5		181.1	7.7	5	
2	175.2	12.1	5		188.1	18.0	5		172.2	25.1	5		191.2	9.5	5		187.2	8.2	5	
3	173.7	12.5	5		182.1	9.3	5		174.5	6.1	5		187.9	10.7	5		186.3	14.5	5	
4	169.3	12.0	5		185.9	11.3	5		178.8	16.5	5		193.8	12.5	5		196.1	12.4	5	

\* means p<0.05, versus control group

\*\* means p<0.01, versus control group

S.D. = standard deviation N = number of animals

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Food consumption (g)

Group mean values per animal - Day 1 - Day 91

Males

GROUP	DAY 77				DAY 84				DAY 91				TOTAL DAY 1 TO DAY 91			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	179.8	10.1	4		182.6	12.5	5		176.3	12.2	5		2223.0	95.5	2	
2	185.0	12.1	5		190.3	16.0	5		185.1	11.5	5		2385.3	143.1	4	
3	187.3	11.4	5		192.2	14.3	5		191.8	15.6	5		2404.1	136.9	4	
4	196.1	11.4	5		200.6	16.0	5		191.9	19.5	5		2431.0	157.7	5	

p>0.05, versus control group

S.D. = standard deviation N = number of animals

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Food consumption (g)

Group mean values per animal - Day 1 - Day 91

Females

GROUP	DAY 7				DAY 14				DAY 21				DAY 28				DAY 35			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	96.4	3.0	5		118.6	6.0	5		111.4	7.8	4		116.2	9.5	5		119.9	7.7	5	
2	102.1	8.2	5		116.9	8.7	5		125.7	11.7	5		125.4	9.4	5		120.4	7.1	5	
3	102.9	10.4	5		126.3	11.4	3		126.8	8.2	5		126.1	9.3	5		126.8	12.2	5	
4	108.7	8.6	5		127.9	4.1	5		120.5	7.9	5		128.9	3.8	5		126.5	7.6	4	

GROUP	DAY 42				DAY 49				DAY 56				DAY 63				DAY 70			
	Mean	S.D.	N	p																
1	114.4	6.9	5		117.5	4.8	5		114.3	14.1	5		129.0	9.7	5		126.0	10.9	5	
2	120.9	9.1	5		123.9	11.8	5		116.5	8.5	5		127.7	8.5	5		132.0	7.9	5	
3	116.7	9.8	5		127.0	5.5	5		122.0	8.3	5		130.3	2.7	5		138.9	8.2	5	
4	120.4	4.7	5		125.8	3.1	5		124.2	6.2	5		137.0	8.5	5		138.1	7.9	5	

p>0.05, versus control group

S.D. = standard deviation N = number of animals

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Food consumption (g)

Group mean values per animal - Day 1 - Day 91

Females

GROUP	DAY 77				DAY 84				DAY 91				TOTAL DAY 1 TO DAY 91			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	124.8	7.8	5		127.0	8.8	5		125.4	8.3	5		1547.5	69.5	4	
2	132.1	8.2	5		124.4	9.4	5		124.1	6.4	5		1592.1	81.6	5	
3	136.1	4.9	5		128.8	9.3	5		130.0	10.5	5		1616.0	44.7	3	
4	130.4	6.9	5		131.3	4.7	5		134.2	10.6	5		1649.4	38.3	4	

p>0.05, versus control group

S.D. = standard deviation N = number of animals

**Table 5 Water consumption – Group mean values**

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Water consumption (g)

Group mean values per animal - Day 1 - Day 91

Males

GROUP	DAY 1-3				DAY 3-7				DAY 7-10				DAY 10-14				DAY 14-17			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	49.6	5.7	5		85.7	10.8	5		60.7	5.7	5		85.5	11.0	5		65.6	7.0	5	
2	65.9	26.6	5		96.5	7.9	4		63.9	5.8	5		92.2	4.9	5		74.2	7.3	5	
3	63.3	20.3	5		80.6	14.4	4		59.4	2.8	4		87.0	13.4	5		65.5	8.8	4	
4	51.0	2.4	5		90.6	3.6	5		65.6	8.6	4		85.8	6.9	5		77.4	6.2	5	

GROUP	DAY 17-21				DAY 21-24				DAY 24-28				DAY 28-31				DAY 31-35			
	Mean	S.D.	N	p																
1	83.8	14.5	5		63.8	7.5	5		83.7	10.0	5		58.1	9.9	5		83.4	12.6	4	
2	85.4	5.7	5		66.9	6.9	5		57.4	40.5	5		71.1	14.5	5		82.6	9.6	4	
3	91.3	4.4	5		62.3	4.3	5		86.8	4.8	4		63.3	8.7	4		86.3	2.5	5	
4	90.8	4.0	5		73.2	6.4	5		95.3	4.6	4		69.7	8.9	5		92.8	6.6	5	

p>0.05, versus control group

S.D. = standard deviation N = number of animals

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Water consumption (g)

Group mean values per animal - Day 1 - Day 91

Males

GROUP	DAY 35-38				DAY 38-42				DAY 42-45				DAY 45-49				DAY 49-52			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	62.1	5.8	4		83.1	11.0	5		57.1	3.2	5		79.5	10.8	5		84.4	5.9	5	
2	62.5	9.3	5		106.1	11.9	5	*	64.3	7.0	5		87.8	12.8	5		82.2	8.7	5	
3	65.9	12.5	5		102.3	9.5	4		65.1	5.7	5		88.8	3.7	5		85.9	6.1	5	
4	73.7	3.3	5		114.7	18.8	5	**	68.5	3.9	5	**	98.8	6.5	5	*	94.1	2.6	5	

GROUP	DAY 52-56				DAY 56-59				DAY 59-63				DAY 63-66				DAY 66-70			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	123.3	5.3	5		98.9	9.7	5		132.1	11.0	5		88.6	7.9	5		126.5	12.2	5	
2	113.8	25.2	5		102.7	4.6	5		136.2	9.5	5		87.2	5.0	5		130.5	8.4	4	
3	129.1	6.2	5		97.3	5.1	5		135.6	8.3	5		83.9	11.2	5		134.1	9.7	5	
4	162.5	43.0	5		124.9	28.6	5	*	159.3	8.8	5	**	100.5	4.7	5		150.5	5.9	5	**

\* means p<0.05, versus control group

\*\* means p<0.01, versus control group

S.D. = standard deviation N = number of animals

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Water consumption (g)

Group mean values per animal - Day 1 - Day 91

Males

GROUP	DAY 70-73				DAY 73-77				DAY 77-80				DAY 80-84				DAY 84-87			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	93.0	9.7	5		108.6	13.4	5		93.2	12.7	5		116.2	11.6	5		92.9	2.4	4	
2	93.8	13.6	5		119.7	7.2	5		96.1	6.5	4		120.2	12.3	5		91.8	6.3	5	
3	97.0	7.6	5		125.4	6.5	5	*	101.4	7.6	5		127.0	10.2	5		96.6	8.1	5	
4	111.6	1.8	5	*	142.9	6.9	5	**	113.8	5.8	5	**	150.7	8.3	5	**	113.1	5.3	5	**

GROUP	DAY 87-91				TOTAL, DAY 1 TO DAY 91			
	Mean	S.D.	N	p	Mean	S.D.	N	p
1	123.5	13.3	5		2425.0	256.0	2	
2	125.1	11.4	5		2455.3	138.9	2	
3	131.1	11.9	5		2270.0		1	
4	139.9	32.7	5		2686.0	122.8	3	

\* means p<0.05, versus control group

\*\* means p<0.01, versus control group

S.D. = standard deviation N = number of animals

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Water consumption (g)

Group mean values per animal - Day 1 - Day 91

Females

GROUP	DAY 1-3				DAY 3-7				DAY 7-10				DAY 10-14				DAY 14-17			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	43.5	5.5	5		64.4	6.1	5		51.8	7.8	5		74.5	9.8	5		55.1	9.5	5	
2	45.9	9.6	5		74.7	17.2	5		50.7	9.3	5		76.1	13.9	5		57.8	17.0	5	
3	68.9	30.8	4		75.3	7.8	5	*	55.9	12.6	4		75.1	9.2	5		64.9	8.6	5	
4	43.2	4.0	5		75.9	6.1	5	*	55.3	4.1	5		71.7	7.0	5		61.6	6.8	5	

GROUP	DAY 17-21				DAY 21-24				DAY 24-28				DAY 28-31				DAY 31-35			
	Mean	S.D.	N	p																
1	83.1	9.2	5		55.5	8.7	4		74.8	9.5	4		59.8	9.1	5		78.0	12.7	5	
2	86.9	14.7	5		58.6	7.3	5		81.5	9.5	5		60.6	9.9	5		80.0	14.5	5	
3	94.2	4.5	5		57.6	5.0	5		88.8	14.2	5		66.9	8.9	4		88.1	14.4	5	
4	78.9	12.7	5		58.9	5.2	4		83.3	4.3	4		65.5	5.4	5		83.7	3.7	5	

\* means 0.01<p<0.05, versus control group

S.D. = standard deviation N = number of animals

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Water consumption (g)

Group mean values per animal - Day 1 - Day 91

Females

GROUP	DAY 35-38				DAY 38-42				DAY 42-45				DAY 45-49				DAY 49-52			
	Mean	S.D.	N	p																
1	58.7	13.1	5		78.2	9.0	5		54.8	5.5	5		78.5	15.7	5		69.1	13.5	5	
2	71.8	17.4	5		95.8	17.5	5		57.6	11.8	5		79.3	15.9	4		71.1	14.7	5	
3	65.1	3.7	5		94.2	10.0	5		62.8	9.2	5		85.8	9.3	5		68.9	5.5	5	
4	63.7	7.9	5		104.2	10.5	5	*	59.3	5.5	5		83.3	6.9	5		75.3	4.0	4	

GROUP	DAY 52-56				DAY 56-59				DAY 59-63				DAY 63-66				DAY 66-70			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	99.1	8.5	4		86.3	12.6	5		115.7	13.2	5		75.5	10.7	5		103.1	11.9	5	
2	99.5	16.8	4		88.5	14.1	5		121.1	19.7	5		74.4	11.0	5		114.9	17.0	5	
3	115.2	15.9	5		92.5	10.4	5		124.9	9.7	5		78.0	7.9	5		128.6	10.1	4	
4	118.2	7.5	5	**	97.2	2.5	5		129.8	7.0	5		81.4	6.2	5		120.4	11.5	4	

\* means p<0.05, versus control group

\*\* means p<0.01, versus control group

S.D. = standard deviation N = number of animals

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Water consumption (g)

Group mean values per animal - Day 1 - Day 91

Females

GROUP	DAY 70-73				DAY 73-77				DAY 77-80				DAY 80-84				DAY 84-87			
	Mean	S.D.	N	p																
1	77.1	12.2	5		96.0	9.0	5		71.9	9.8	5		103.3	12.5	5		78.4	7.5	5	
2	82.1	14.3	4		104.3	11.2	5		74.6	16.2	5		106.0	11.0	5		79.2	7.3	5	
3	85.8	7.5	5		111.8	6.8	5	*	80.5	10.2	5		100.6	4.4	5		82.3	11.9	5	
4	85.7	8.5	5		112.4	7.1	5	*	82.9	3.0	5		116.6	13.9	5		86.1	6.8	5	

GROUP	DAY 87-91				TOTAL, DAY 1 TO DAY 91			
	Mean	S.D.	N	p	Mean	S.D.	N	p
1	101.9	13.1	5		2133.0	135.0	3	
2	111.3	23.8	5		1918.3	231.2	3	
3	119.0	15.9	5		2206.8	271.9	2	
4	118.2	9.4	5		2219.0		1	

\* means 0.01<p<0.05, versus control group

S.D. = standard deviation N = number of animals

**Table 6 Haematology – Group mean values**

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Haematology

Group mean values

Males

GROUP	Hb				RBC				HT				MCV				MCH			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	10.19	0.84	10		9.63	0.40	10		48.8	2.1	10		50.9	1.6	10		1.06	0.07	10	
2	10.39	0.23	9		9.51	0.22	9		48.6	1.0	9		51.1	0.9	9		1.10	0.00	9	
3	10.43	0.34	9		9.49	0.33	9		49.0	1.2	9		51.6	1.9	9		1.11	0.06	9	
4	10.57	0.30	10		9.52	0.32	10		49.8	1.4	10		52.3	1.8	10		1.11	0.06	10	

GROUP	MCHC				WBC				% NEUTRO				NEUTRO				% LYMPHO			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	20.8	1.4	10		10.28	2.40	10		6.6	2.3	10		0.68	0.23	10		89.9	2.6	10	
2	21.4	0.2	9		10.74	1.32	9		6.1	2.5	9		0.68	0.31	9		90.2	2.6	9	
3	21.3	0.3	9		9.89	2.35	9		7.9	2.8	9		0.78	0.32	9		87.2	2.7	9	
4	21.2	0.3	10		12.78	3.43	10		8.5	3.3	10		1.10	0.55	10		87.6	3.5	10	

Abbreviations and units are explained in subsection 'Clinical Pathology'

p>0.05, versus control group

S.D. = standard deviation N = number of animals

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Haematology

Group mean values

Males

GROUP	LYMPHO				% EOS				EOS				% BASO				BASO			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	9.20	2.15	10		1.8	0.6	10		0.20	0.08	10		0.0	0.0	10		0.00	0.00	10	
2	9.68	1.05	9		1.9	0.6	9		0.22	0.08	9		0.0	0.0	9		0.00	0.00	9	
3	8.63	2.09	9		1.9	0.6	9		0.18	0.04	9		0.2	0.4	9		0.03	0.07	9	
4	11.18	3.10	10		1.9	0.6	10		0.24	0.10	10		0.1	0.3	10		0.03	0.05	10	

GROUP	% MONO				MONO				Plt				APTT				Pt			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	1.7	1.6	10		0.18	0.19	10		598	294	10		19.7	4.1	10		16.3	1.1	10	
2	1.8	0.8	9		0.17	0.09	9		781	55	9		16.6	2.6	9		16.3	0.8	9	
3	2.8	1.9	9		0.27	0.17	9		706	111	9		16.3	2.8	9		16.2	0.8	9	
4	2.0	0.9	10		0.25	0.14	10		704	74	10		17.5	2.6	10		15.9	0.8	10	

Abbreviations and units are explained in subsection 'Clinical Pathology'

p>0.05, versus control group

S.D. = standard deviation N = number of animals

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Haematology

Group mean values

Males

GROUP	Fib			
	Mean	S.D.	N	p
1	3.02	0.42	10	
2	3.17	0.25	9	
3	3.21	0.25	9	
4	3.12	0.36	10	

Abbreviations and units are explained in subsection 'Clinical Pathology'

p>0.05, versus control group

S.D. = standard deviation N = number of animals

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Haematology

Group mean values

Females

GROUP	Hb				RBC				HT				MCV				MCH			
	Mean	S.D.	N	p																
1	9.80	0.27	10		8.77	0.23	10		45.7	1.3	10		52.4	0.8	10		1.12	0.04	10	
2	9.58	0.20	10		8.49	0.26	10		44.7	1.2	10		52.4	1.3	10		1.12	0.04	10	
3	9.98	0.39	8		8.74	0.29	8		46.3	2.0	8		53.1	1.8	8		1.15	0.05	8	
4	9.69	0.30	10		8.59	0.28	10		45.4	1.5	10		53.0	1.8	10		1.14	0.05	10	

GROUP	MCHC				WBC				% NEUTRO				NEUTRO				% LYMPHO			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	21.4	0.3	10		8.91	2.23	10		5.8	1.6	10		0.51	0.21	10		91.0	1.8	10	
2	21.5	0.3	10		8.13	3.11	9		6.0	1.6	10		0.51	0.23	10		91.0	2.4	10	
3	21.6	0.2	8		7.14	0.45	7	*	6.5	1.3	8		0.48	0.09	8		90.0	1.6	8	
4	21.3	0.3	10		7.38	1.97	10		7.2	2.5	10		0.52	0.21	10		89.2	3.5	10	

Abbreviations and units are explained in subsection 'Clinical Pathology'

\* means 0.01<p<0.05, versus control group

S.D. = standard deviation N = number of animals

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Haematology

Group mean values

Females

GROUP	LYMPHO				% EOS				EOS				% BASO				BASO			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	8.11	2.08	10		1.9	0.6	10		0.18	0.04	10		0.3	0.5	10		0.02	0.04	10	
2	7.45	2.67	10		1.9	0.6	10		0.15	0.05	10		0.5	0.5	10		0.03	0.05	10	
3	6.71	0.85	8		2.5	0.5	8		0.19	0.06	8		0.1	0.4	8		0.00	0.00	8	
4	6.60	1.90	10		3.1	1.8	10		0.19	0.09	10		0.3	0.5	10		0.02	0.04	10	

GROUP	% MONO				MONO				Plt				APTT				Pt			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	1.2	0.6	10		0.10	0.05	10		804	65	10		15.3	1.4	10		16.7	0.8	10	
2	0.8	0.4	10		0.06	0.05	10		799	75	10		17.1	4.2	8		16.5	1.0	10	
3	1.1	0.4	8		0.09	0.04	8		815	50	8		17.4	7.6	9		16.0	0.5	8	
4	0.7	0.5	10		0.05	0.05	10		804	117	10		15.5	0.7	10		16.2	0.7	10	

Abbreviations and units are explained in subsection 'Clinical Pathology'

p>0.05, versus control group

S.D. = standard deviation N = number of animals

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Haematology

Group mean values

Females

GROUP	Fib			
	Mean	S.D.	N	p
1	2.51	0.29	10	
2	2.52	0.19	10	
3	2.26	0.45	9	
4	2.63	0.16	10	

Abbreviations and units are explained in subsection 'Clinical Pathology'

p>0.05, versus control group

S.D. = standard deviation N = number of animals

**Table 7 Clinical chemistry – Group mean values**

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Clinical chemistry

Group mean values

Males

GROUP	ALAT				ASAT				ALKPH				BILI				GGT			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	1.16	0.19	10		1.74	0.49	10		2.54	0.46	10		<1.31	>0.03	10		<0.04	>0.00	10	
2	1.13	0.19	9		1.72	0.32	9		2.71	0.19	9		<1.31	>0.03	9		<0.04	>0.00	9	
3	1.06	0.25	8		1.68	0.29	8		2.93	0.47	8		<1.32	>0.04	8		<0.04	>0.00	8	
4	1.01	0.22	10		1.80	0.30	10		3.10	0.35	10	**	<1.31	>0.03	10		<0.04	>0.00	10	

GROUP	CHOL				TRIG				UREA				CREAT				GLUC			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	2.37	0.37	10		1.62	0.58	10		6.94	0.90	10		29.1	2.7	10		6.81	1.23	10	
2	2.34	0.21	9		1.68	0.31	9		7.22	0.57	9		26.9	2.0	9		6.21	0.59	9	
3	2.14	0.34	8		1.36	0.44	8		7.40	0.97	8		27.3	2.1	8		6.25	0.28	8	
4	2.17	0.25	10		1.45	0.44	10		7.57	0.71	10		27.7	2.8	10		6.09	0.38	10	

Abbreviations and units are explained in subsection 'Clinical Pathology'

Limit of detection for BILI is 1.3 - this value is used in the calculation

Limit of detection for GGT is 0.04 - this value is used in the calculation

\*\* means p<0.01, versus control group

S.D. = standard deviation N = number of animals

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Clinical chemistry

Group mean values

Males

GROUP	Na				K				Ca				Mg				P			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	143.4	1.9	10		5.93	0.60	10		2.80	0.10	10		0.94	0.05	10		2.39	0.13	10	
2	143.7	1.5	9		6.08	0.87	9		2.77	0.07	9		0.93	0.06	9		2.33	0.17	9	
3	143.7	1.9	8		5.87	0.80	8		2.77	0.06	8		0.90	0.06	8		2.19	0.18	8	
4	142.1	1.6	10		6.55	0.86	10		2.73	0.12	10		0.95	0.04	10		2.19	0.22	10	

GROUP	CL				PROTEIN				ALB				GLOBULIN				ALB/G Ratio			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	100.8	1.4	10		72.5	5.1	10		46.2	2.8	10		26.3	2.9	10		1.77	0.14	10	
2	100.8	0.6	9		70.9	2.7	9		45.2	1.8	9		25.7	1.4	9		1.77	0.10	9	
3	101.0	1.6	8		70.5	3.1	8		46.0	1.8	8		24.5	2.1	8		1.89	0.16	8	
4	99.9	1.3	10		71.6	2.4	10		45.6	1.2	10		26.0	1.7	10		1.76	0.11	10	

Abbreviations and units are explained in subsection 'Clinical Pathology'

p>0.05, versus control group

S.D. = standard deviation N = number of animals

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Clinical chemistry

Group mean values

Females

GROUP	ALAT				ASAT				ALKPH				BILI				GGT			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	0.94	0.11	10		1.79	0.20	10		1.94	0.34	10		<1.38	>0.14	10		<0.04	>0.00	10	
2	0.78	0.10	10	**	1.53	0.34	10		1.79	0.35	10		<1.46	>0.18	10		<0.04	>0.00	10	
3	0.77	0.08	8	**	1.56	0.25	8		1.98	0.43	8		<1.40	>0.14	8		<0.04	>0.00	8	
4	0.76	0.10	10	**	1.50	0.14	10	*	2.03	0.37	10		<1.56	>0.58	10		<0.04	>0.00	10	

GROUP	CHOL				TRIG				UREA				CREAT				GLUC			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	2.34	0.36	10		0.58	0.22	10		6.47	0.82	10		29.5	3.2	10		6.70	0.90	10	
2	2.67	0.41	10		0.65	0.24	10		7.08	0.97	10		30.5	2.4	10		6.31	0.68	10	
3	2.35	0.26	8		0.59	0.34	8		6.33	0.69	8		29.3	2.5	8		6.20	0.45	8	
4	2.48	0.42	10		0.63	0.23	10		7.26	1.29	10		31.0	2.2	10		6.34	0.82	10	

Abbreviations and units are explained in subsection 'Clinical Pathology'

Limit of detection for BILI is 1.3 - this value is used in the calculation  
Limit of detection for GGT is 0.04 - this value is used in the calculation

\* means p<0.05, versus control group

\*\* means p<0.01, versus control group

S.D. = standard deviation N = number of animals

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Clinical chemistry

Group mean values

Females

GROUP	Na				K				Ca				Mg				P			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	142.4	2.0	10		6.07	0.75	10		2.72	0.09	10		1.00	0.09	10		1.93	0.41	10	
2	142.3	1.8	10		5.72	0.61	10		2.73	0.09	10		0.98	0.05	10		1.90	0.32	10	
3	141.9	1.8	8		6.07	0.49	8		2.71	0.08	8		0.97	0.06	8		1.80	0.36	8	
4	141.4	1.7	10		6.27	0.50	10		2.76	0.09	10		0.99	0.09	10		1.90	0.41	10	

GROUP	CL				PROTEIN				ALB				GLOBULIN				ALB/G Ratio			
	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p	Mean	S.D.	N	p
1	102.3	2.1	10		70.0	3.5	10		48.2	2.3	10		21.8	1.9	10		2.23	0.19	10	
2	101.9	1.4	10		69.6	3.2	10		48.0	2.8	10		21.6	1.5	10		2.24	0.20	10	
3	101.9	1.4	8		70.7	2.9	8		49.4	2.6	8		21.3	1.4	8		2.33	0.20	8	
4	101.8	1.3	10		71.4	3.8	10		49.2	2.9	10		22.2	1.9	10		2.23	0.19	10	

Abbreviations and units are explained in subsection 'Clinical Pathology'

p>0.05, versus control group

S.D. = standard deviation N = number of animals

**Table 8 Organ weight – Group mean values**

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Absolute (mg) and relative (% of body wt and brain wt) organ weight

Group mean values

Males

GROUP	BODY WT, g				ADRENALS				ADRENALS				ADRENALS				BRAIN			
	BODY WT, g				ABSOLUTE				RELATIVE				% OF BRAIN WT				ABSOLUTE			
	MEAN	S.D.	N	p	MEAN	S.D.	N	p	MEAN	S.D.	N	p	MEAN	S.D.	N	p	MEAN	S.D.	N	p
1	450.8	36.3	10		57.0	11.3	10		0.0126	0.0023	10		2.60	0.53	10		2201	71	10	
2	451.9	29.2	9		57.8	9.7	9		0.0127	0.0015	9		2.60	0.38	9		2214	89	9	
3	441.1	22.0	9		61.9	10.8	9		0.0140	0.0023	9		2.82	0.54	9		2207	90	9	
4	445.9	24.2	10		53.4	9.3	10		0.0120	0.0023	10		2.43	0.45	10		2201	95	10	

GROUP	BRAIN				EPIDIDYIMIDES				EPIDIDYIMIDES				EPIDIDYIMIDES				HEART			
	RELATIVE				ABSOLUTE				RELATIVE				% OF BRAIN WT				ABSOLUTE			
	MEAN	S.D.	N	p	MEAN	S.D.	N	p	MEAN	S.D.	N	p	MEAN	S.D.	N	p	MEAN	S.D.	N	p
1	0.491	0.044	10		1370	128	10		0.305	0.028	10		62.3	5.7	10		1550	147	10	
2	0.491	0.028	9		1365	131	9		0.303	0.029	9		61.6	5.5	9		1510	101	9	
3	0.502	0.034	9		1396	62	9		0.317	0.020	9		63.3	4.1	9		1533	119	9	
4	0.495	0.033	10		1327	123	10		0.298	0.030	10		60.4	6.2	10		1518	134	10	

p>0.05, versus control group

S.D. = standard deviation N = number of animals

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Absolute (mg) and relative (% of body wt and brain wt) organ weight

Group mean values

Males

GROUP	HEART				HEART				KIDNEYS				KIDNEYS				KIDNEYS			
	RELATIVE				% OF BRAIN WT				ABSOLUTE				RELATIVE				% OF BRAIN WT			
	MEAN	S.D.	N	p	MEAN	S.D.	N	p	MEAN	S.D.	N	p	MEAN	S.D.	N	p	MEAN	S.D.	N	p
1	0.344	0.029	10		70.5	6.7	10		2926	180	10		0.651	0.045	10		133.1	10.3	10	
2	0.335	0.020	9		68.2	3.5	9		3059	345	9		0.677	0.060	9		138.2	14.8	9	
3	0.348	0.023	9		69.6	6.4	9		2952	250	9		0.669	0.045	9		134.0	12.7	9	
4	0.340	0.020	10		69.0	6.1	10		2961	311	10		0.664	0.060	10		134.6	13.3	10	

GROUP	LIVER				LIVER				LIVER				SPLEEN				SPLEEN			
	ABSOLUTE				RELATIVE				% OF BRAIN WT				ABSOLUTE				RELATIVE			
	MEAN	S.D.	N	p	MEAN	S.D.	N	p	MEAN	S.D.	N	p	MEAN	S.D.	N	p	MEAN	S.D.	N	p
1	16593	2284	10		3.67	0.21	10		755.6	114.7	10		856	84	10		0.190	0.014	10	
2	16171	1718	9		3.57	0.22	9		730.7	76.7	9		874	93	9		0.193	0.009	9	
3	14868	1369	9		3.37	0.18	9	**	675.6	76.4	9		824	98	9		0.187	0.018	9	
4	15599	1375	10		3.50	0.17	10		710.1	69.8	10		901	149	10		0.203	0.034	10	

\*\* means p<0.01, versus control group

S.D. = standard deviation N = number of animals

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Absolute (mg) and relative (% of body wt and brain wt) organ weight

Group mean values

Males

GROUP	SPLEEN				TESTES				TESTES				TESTES				THYMUS			
	% OF BRAIN WT				ABSOLUTE				RELATIVE				% OF BRAIN WT				ABSOLUTE			
	MEAN	S.D.	N	p	MEAN	S.D.	N	p	MEAN	S.D.	N	p	MEAN	S.D.	N	p	MEAN	S.D.	N	p
1	39.0	4.1	10		3718	209	10		0.829	0.073	10		169.0	9.5	10		462	138	10	
2	39.4	3.3	9		3723	216	9		0.825	0.033	9		168.2	9.1	9		438	128	9	
3	37.4	4.5	9		3847	155	9		0.874	0.059	9		174.7	10.7	9		458	85	9	
4	41.0	6.9	10		3737	223	10		0.841	0.077	10		170.1	12.8	10		499	124	10	

GROUP	THYMUS				THYMUS			
	RELATIVE				% OF BRAIN WT			
	MEAN	S.D.	N	p	MEAN	S.D.	N	p
1	0.103	0.029	10		20.9	6.0	10	
2	0.097	0.028	9		19.7	5.6	9	
3	0.104	0.019	9		20.7	3.7	9	
4	0.112	0.026	10		22.7	5.4	10	

p>0.05, versus control group

S.D. = standard deviation N = number of animals

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Absolute (mg) and relative (% of body wt and brain wt) organ weight

Group mean values

Females

GROUP	BODY WT, g				ADRENALS				ADRENALS				ADRENALS				BRAIN			
					ABSOLUTE				RELATIVE				% OF BRAIN WT				ABSOLUTE			
	Mean	S.D	N	p	Mean	S.D	N	p	Mean	S.D	N	p	Mean	S.D	N	p	Mean	S.D	N	p
1	269.7	13.3	10		71.1	11.2	10		0.0264	0.0046	10		3.45	0.56	10		2064	107	10	
2	274.6	9.0	10		71.1	7.7	10		0.0259	0.0025	10		3.43	0.30	10		2072	68	10	
3	275.0	12.5	8		71.4	3.8	8		0.0260	0.0014	8		3.42	0.19	8		2091	58	8	
4	271.1	18.9	10		71.8	7.4	10		0.0266	0.0037	10		3.51	0.37	10		2046	86	10	

GROUP	BRAIN				HEART				HEART				HEART				KIDNEYS			
	RELATIVE				ABSOLUTE				RELATIVE				% OF BRAIN WT				ABSOLUTE			
	Mean	S.D	N	p	Mean	S.D	N	p	Mean	S.D	N	p	Mean	S.D	N	p	Mean	S.D	N	p
1	0.767	0.060	10		1028	73	10		0.382	0.028	10		49.9	4.8	10		1837	67	10	
2	0.755	0.022	10		1050	99	10		0.382	0.035	10		50.7	5.6	10		1895	133	10	
3	0.762	0.047	8		1057	78	8		0.385	0.027	8		50.7	4.9	8		1859	111	8	
4	0.757	0.054	10		1058	96	10		0.391	0.038	10		51.8	5.7	10		1848	199	10	

p>0.05, versus control group

S.D. = standard deviation N = number of animals

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Absolute (mg) and relative (% of body wt and brain wt) organ weight

Group mean values

Females

GROUP	KIDNEYS				KIDNEYS				LIVER				LIVER				LIVER			
	RELATIVE				% OF BRAIN WT				ABSOLUTE				RELATIVE				% OF BRAIN WT			
	Mean	S.D	N	p	Mean	S.D	N	p	Mean	S.D	N	p	Mean	S.D	N	p	Mean	S.D	N	p
1	0.682	0.026	10		89.2	5.6	10		9086	659	10		3.37	0.19	10		441.5	42.7	10	
2	0.690	0.038	10		91.4	4.8	10		9489	1317	10		3.45	0.42	10		457.8	60.9	10	
3	0.678	0.052	8		89.0	5.7	8		8852	450	8		3.22	0.14	8		423.5	21.2	8	
4	0.681	0.039	10		90.4	9.3	10		8745	807	10		3.22	0.13	10		428.2	42.4	10	

GROUP	OVARIES				OVARIES				OVARIES				SPLEEN				SPLEEN			
	ABSOLUTE				RELATIVE				% OF BRAIN WT				ABSOLUTE				RELATIVE			
	Mean	S.D	N	p	Mean	S.D	N	p	Mean	S.D	N	p	Mean	S.D	N	p	Mean	S.D	N	p
1	115.7	21.9	10		0.0429	0.0075	10		5.64	1.24	10		618	61	10		0.230	0.022	10	
2	106.3	18.5	10		0.0388	0.0071	10		5.14	0.93	10		615	97	10		0.224	0.035	10	
3	113.3	17.6	8		0.0413	0.0069	8		5.42	0.84	8		610	73	8		0.222	0.023	8	
4	112.1	24.5	10		0.0415	0.0095	10		5.51	1.33	10		592	78	10		0.218	0.020	10	

p>0.05, versus control group

S.D. = standard deviation N = number of animals

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Absolute (mg) and relative (% of body wt and brain wt) organ weight

Group mean values

Females

GROUP	SPLEEN				THYMUS				THYMUS				THYMUS				UTERUS			
	% OF BRAIN WT				ABSOLUTE				RELATIVE				% OF BRAIN WT				ABSOLUTE			
	Mean	S.D	N	p	Mean	S.D	N	p	Mean	S.D	N	p	Mean	S.D	N	p	Mean	S.D	N	p
1	30.1	4.0	10		324	68	10		0.120	0.023	10		15.8	3.9	10		800	336	10	
2	29.7	4.5	10		286	61	10		0.104	0.022	10		13.8	2.8	10		706	140	10	
3	29.3	4.2	8		281	45	8		0.103	0.017	8		13.5	2.4	8		924	439	8	
4	28.9	3.6	10		313	70	10		0.115	0.020	10		15.3	3.3	10		654	145	10	

GROUP	UTERUS				UTERUS			
	RELATIVE				% OF BRAIN WT			
	Mean	S.D	N	p	Mean	S.D	N	p
1	0.298	0.126	10		39.1	17.8	10	
2	0.257	0.047	10		34.1	6.8	10	
3	0.335	0.156	8		44.5	21.9	8	
4	0.243	0.060	10		32.0	7.3	10	

p>0.05, versus control group

S.D. = standard deviation N = number of animals

**Table 9 Clinical signs – Individual findings**

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Clinical signs

Individual findings

Group 1 - Males

Animal No 1

No adverse clinical signs.

Animal No 2

No adverse clinical signs.

Animal No 3

No adverse clinical signs.

Animal No 4

No adverse clinical signs.

Animal No 5

No adverse clinical signs.

Animal No 6

No adverse clinical signs.

Animal No 7

No adverse clinical signs.

Animal No 8

No adverse clinical signs.

Animal No 9

No adverse clinical signs.

Animal No 10

No adverse clinical signs.

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Clinical signs

Individual findings

Group 1 - Females

Animal No 11

No adverse clinical signs.

Animal No 12

No adverse clinical signs.

Animal No 13

No adverse clinical signs.

Animal No 14

No adverse clinical signs.

Animal No 15

No adverse clinical signs.

Animal No 16

Days 43-64: Hairless areas on the forelegs.  
Days 65-87: Thin-haired areas on the forelegs.

Animal No 17

No adverse clinical signs.

Animal No 18

No adverse clinical signs.

Animal No 19

No adverse clinical signs.

Animal No 20

No adverse clinical signs.

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Clinical signs

Individual findings

Group 2 - Males

Animal No 21

No adverse clinical signs.  
Day 33: Found dead.

Animal No 22

No adverse clinical signs.

Animal No 23

Day 35-91: Hairless areas on the forelegs.

Animal No 24

No adverse clinical signs.

Animal No 25

No adverse clinical signs.

Animal No 26

No adverse clinical signs.

Animal No 27

No adverse clinical signs.

Animal No 28

No adverse clinical signs.

Animal No 29

No adverse clinical signs.

Animal No 30

No adverse clinical signs.

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Clinical signs

Individual findings

Group 2 - Females

Animal No 31

No adverse clinical signs.

Animal No 32

No adverse clinical signs.

Animal No 33

No adverse clinical signs.

Animal No 34

Day 26: Blood on the gavage after dosing.

Animal No 35

No adverse clinical signs.

Animal No 36

No adverse clinical signs.

Animal No 37

No adverse clinical signs.

Animal No 38

No adverse clinical signs.

Animal No 39

No adverse clinical signs.

Animal No 40

No adverse clinical signs.

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Clinical signs

Individual findings

Group 3 - Males

Animal No 41

No adverse clinical signs.

Animal No 42

Days 23-24: Red discharge around the right eye.  
Day 25: Found dead.

Animal No 43

No adverse clinical signs.

Animal No 44

No adverse clinical signs.

Animal No 45

No adverse clinical signs.

Animal No 46

No adverse clinical signs.

Animal No 47

No adverse clinical signs.

Animal No 48

No adverse clinical signs.

Animal No 49

No adverse clinical signs.

Animal No 50

No adverse clinical signs.

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Clinical signs

Individual findings

Group 3 - Females

Animal No 51

Day 7 Blood on the gavage after dosing.  
Laboured respiration. Passive.  
Lacklustre eyes.  
At 10:55 h: Found dead.

Animal No 56

No adverse clinical signs.

Animal No 57

No adverse clinical signs.

Animal No 52

Days 71-91: Hairless on the forelegs.

Animal No 58

No adverse clinical signs.

Animal No 53

No adverse clinical signs.

Animal No 59

No adverse clinical signs.

Animal No 54

No adverse clinical signs.

Animal No 60

No adverse clinical signs.

Animal No 55

Day 8: 15 min after dosing: Passive. Laboured  
respiration. Lacklustre eyes.  
30 min after dosing: Pale tail. Poor  
balance. Laboured respiration.  
Lying down passively. Lacklustre  
eyes. Pale tail.  
Flaccid and moribund during blood  
sampling.  
Sent for necropsy.

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Clinical signs

Individual findings

Group 4 - Males

Animal No 61

Day 12: Red around the snout.

Animal No 66

No adverse clinical signs.

Animal No 62

No adverse clinical signs.

Animal No 67

No adverse clinical signs.

Animal No 63

No adverse clinical signs.

Animal No 68

Day 12: Red discoloration around the snout.

Animal No 64

No adverse clinical signs.

Animal No 69

No adverse clinical signs.

Animal No 65

No adverse clinical signs.

Animal No 70

No adverse clinical signs.

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Clinical signs

Individual findings

Group 3 - Females

Animal No 71

Day 91: Wound on the left side of the anterior part of the back.

Animal No 76

No adverse clinical signs.

Animal No 72

No adverse clinical signs.

Animal No 77

No adverse clinical signs.

Animal No 73

No adverse clinical signs.

Animal No 78

No adverse clinical signs.

Animal No 74

No adverse clinical signs.

Animal No 79

No adverse clinical signs.

Animal No 75

No adverse clinical signs.

Animal No 70

No adverse clinical signs.

**Table 10 Open field testing – Individual values**

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Open field testing

Individual values

Males

GROUP	ANIMAL NO	TIME MOVING (s)	TOTAL DISTANCE (m)	NO. OF REARINGS	TIME CENTRE (s)	TIME PERIPHERY (s)	TOTAL CORNER VISITS	MOVES/COUNTS	FAECES
1	1	220	27.3	26	74	226	15	1099	5
	2	250	38.1	30	26	274	25	1252	1
	3	256	42.7	24	25	275	23	1281	0
	4	253	50.2	44	14	286	31	1263	0
	5	243	34.8	41	20	280	21	1216	1
	6	242	38.9	39	66	234	12	1212	5
	7	233	32.9	26	18	282	15	1167	0
	8	248	42.3	36	17	283	25	1240	1
	9	211	28.3	26	9	291	19	1057	2
	10	238	35.3	28	3	297	24	1189	2
2	21	d							
	22	248	42.3	27	15	285	27	1241	0
	23	249	32.1	30	37	263	16	1244	4
	24	248	43.9	27	25	275	26	1238	0
	25	223	30.5	16	19	281	20	1114	3
	26	245	38.3	39	23	277	21	1227	0
	27	226	37.8	21	26	274	15	1129	0
	28	248	41.0	34	16	284	24	1240	0
	29	232	41.5	38	14	286	22	1158	0
	30	248	46.1	29	21	279	34	1239	0

d = dead before termination of treatment

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Open field testing

Individual values

Males

GROUP	ANIMAL NO	TIME MOVING (s)	TOTAL DISTANCE (m)	NO. OF REARINGS	TIME CENTRE (s)	TIME PERIPHERY (s)	TOTAL CORNER VISITS	MOVES/COUNTS	FAECES
3	41	247	39.1	36	34	266	16	1234	0
	42	d							
	43	233	31.3	58	27	273	18	1166	2
	44	246	40.6	37	13	287	21	1229	0
	45	254	48.1	55	9	291	30	1271	0
	46	246	47.1	82	23	277	25	1230	3
	47	247	44.2	37	27	273	21	1234	0
	48	237	42.4	31	12	288	26	1183	0
	49	228	32.2	44	9	291	17	1138	0
	50	241	33.2	25	6	294	17	1206	0
4	61	254	39.0	21	30	270	18	1272	3
	62	245	39.1	39	21	279	18	1225	0
	63	249	43.3	31	26	274	20	1247	0
	64	249	37.1	39	24	276	19	1246	2
	65	239	39.2	43	22	278	23	1196	3
	66	257	50.9	39	14	286	32	1285	4
	67	234	36.2	37	18	282	19	1171	0
	68	248	42.4	40	14	286	25	1239	0
	69	240	35.9	26	16	284	18	1198	1
	70	211	27.8	22	8	292	18	1057	5

d = dead before termination of treatment

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Open field testing

Individual values

Females

GROUP	ANIMAL NO	TIME MOVING (s)	TOTAL DISTANCE (m)	NO. OF REARINGS	TIME CENTRE (s)	TIME PERIPHERY (s)	TOTAL CORNER VISITS	MOVES/COUNTS	FAECES
1	11	239	51.2	24	10	290	37	1193	0
	12	246	44.9	52	8	292	26	1232	0
	13	251	51.3	21	14	286	31	1253	0
	14	150	20.4	7	10	290	12	748	4
	15	249	51.5	78	14	286	34	1244	0
	16	232	43.6	60	3	297	26	1158	0
	17	255	52.9	48	5	295	32	1277	0
	18	252	60.3	55	9	291	17	1261	0
	19	242	50.6	42	4	296	38	1209	0
	20	235	40.8	48	15	285	19	1176	0
2	31	247	47.8	14	7	293	33	1234	0
	32	256	60.7	31	9	291	50	1279	0
	33	247	52.7	44	7	293	27	1237	0
	34	248	43.7	53	4	296	28	1242	0
	35	236	51.2	57	4	296	38	1178	0
	36	225	35.2	30	8	292	23	1125	0
	37	242	38.9	39	8	292	23	1210	0
	38	250	47.5	45	9	291	15	1250	0
	39	250	46.1	42	2	298	28	1248	0
	40	240	42.8	38	2	298	27	1202	0

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Open field testing

Individual values

Females

GROUP	ANIMAL NO	TIME MOVING (s)	TOTAL DISTANCE (m)	NO. OF REARINGS	TIME CENTRE (s)	TIME PERIPHERY (s)	TOTAL CORNER VISITS	MOVES/COUNTS	FAECES
3	51	d							
	52	241	46.1	26	2	298	28	1203	0
	53	245	43.9	53	10	290	24	1226	0
	54	237	47.0	50	8	292	22	1186	0
	55	d							
	56	246	55.2	37	6	294	34	1229	0
	57	243	38.6	44	0	300	25	1213	0
	58	247	46.4	27	8	292	30	1234	0
	59	228	37.8	35	5	295	24	1142	0
	60	238	45.5	42	3	297	26	1188	0
4	71	248	49.3	44	19	281	26	1240	0
	72	244	43.0	46	1	299	19	1222	0
	73	248	50.3	46	9	291	30	1238	0
	74	238	46.7	41	6	294	27	1191	1
	75	247	48.6	38	1	299	28	1233	0
	76	49	4.4	0	4	296	3	246	4
	77	244	42.3	31	4	296	28	1219	0
	78	242	40.9	53	3	297	22	1210	0
	79	246	44.7	41	10	290	29	1229	0
	80	242	50.5	40	9	291	27	1212	0

d = dead before termination of treatment

**Table 11 Stimuli induced clinical observations – Individual values**

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Stimuli-induced clinical observations

Individual values

Males

GROUP	ANIMAL NO	TOE		GRASP RESPONSE	GRIP STRENGTH	EYELID REFLEX	STARTLE RESPONSE	HEAD SHAKE RESPONSE	RIGHTING REFLEX TABLE	RIGHTING REFLEX HAND	PLACING REFLEX	NEGA-TIVE GEOTAXIS
		PUPIL REFLEX	PINCH REACT.									
1	1	1	1	1	1	1	1	1	1	1	1	1
	2	1	1	1	1	1	1	1	1	1	1	1
	3	1	1	1	1	1	1	1	1	1	1	0
	4	1	1	1	1	1	1	1	1	1	1	1
	5	1	1	1	1	1	1	1	1	1	1	1
	6	1	1	1	1	1	1	1	1	1	1	1
	7	1	1	1	1	1	1	1	1	1	1	1
	8	1	1	1	1	1	1	1	1	1	1	1
	9	1	1	1	1	1	1	1	1	1	1	1
	10	1	1	1	1	1	1	1	1	1	1	1
2	21	d										
	22	1	1	1	1	1	1	1	1	1	1	1
	23	1	1	1	1	1	1	1	1	1	1	1
	24	1	1	1	1	1	1	1	1	1	1	1
	25	1	1	1	1	1	1	1	1	1	1	1
	26	1	1	1	1	1	1	1	1	1	1	1
	27	1	1	1	1	1	1	1	1	1	1	1
	28	1	1	1	1	1	1	1	1	1	1	1
	29	1	1	1	1	1	1	1	1	1	1	1
	30	1	1	1	1	1	1	1	1	1	1	1

d = dead before termination of treatment

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Stimuli-induced clinical observations

Individual values

Males

GROUP	ANIMAL NO	TOE		GRASP RESPONSE	GRIP STRENGTH	EYELID REFLEX	STARTLE RESPONSE	HEAD SHAKE RESPONSE	RIGHTING REFLEX TABLE	RIGHTING REFLEX HAND	PLACING REFLEX	NEGA-TIVE GEOTAXIS
		PUPIL REFLEX	PINCH REACT.									
3	41	1	1	1	1	1	1	1	1	1	1	1
	42	d										
	43	1	1	0	1	1	1	1	1	1	1	1
	44	1	1	1	1	1	1	1	1	1	1	1
	45	1	1	1	1	1	1	1	1	1	1	1
	46	1	1	1	1	1	1	1	1	1	1	1
	47	1	1	1	1	1	1	1	1	1	1	1
	48	1	1	1	1	1	1	1	1	1	1	1
	49	1	1	1	1	1	1	1	1	1	1	1
	50	0	1	1	1	1	1	1	1	1	1	1
4	61	1	1	1	1	1	1	1	1	1	1	1
	62	1	1	1	1	1	1	1	1	1	1	1
	63	1	1	1	1	1	1	1	1	1	1	1
	64	1	1	0	1	1	1	1	1	1	1	1
	65	1	1	1	1	1	1	1	1	1	1	1
	66	1	1	1	1	1	1	1	1	1	1	1
	67	1	1	1	1	1	1	1	1	1	1	0
	68	1	1	1	1	1	1	1	1	1	1	1
	69	1	1	1	1	1	1	1	1	1	1	1
	70	1	1	1	1	1	1	1	1	1	1	0

d = dead before termination of treatment



Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Stimuli-induced clinical observations

Individual values

Females

GROUP	ANIMAL NO	TOE		GRASP RESPONSE	GRIP STRENGTH	EYELID REFLEX	STARTLE RESPONSE	HEAD SHAKE RESPONSE	RIGHTING REFLEX TABLE	RIGHTING REFLEX HAND	PLACING REFLEX	NEGA-TIVE GEOTAXIS
		PUPIL REFLEX	PINCH REACT.									
3	51	d										
	52	1	1	1	1	1	1	1	1	1	1	1
	53	e										
	54	1	1	1	1	1	1	1	1	1	1	1
	55	d										
	56	1	1	1	1	1	1	1	1	1	1	1
	57	1	1	1	1	1	1	1	1	1	1	1
	58	1	1	1	1	1	1	1	1	1	1	1
	59	1	1	1	1	1	1	1	1	1	1	1
	60	1	1	1	1	1	1	1	1	1	1	1
4	71	1	1	1	1	1	1	1	1	1	1	1
	72	1	1	1	1	1	1	1	1	1	1	1
	73	1	1	1	1	1	1	1	1	1	1	1
	74	1	1	1	1	1	1	1	1	1	1	1
	75	1	1	1	1	1	1	1	1	1	1	1
	76	1	1	0	1	1	1	1	1	1	1	1
	77	1	1	1	1	1	1	1	1	1	1	1
	78	1	1	1	1	1	1	1	1	1	1	1
	79	1	1	1	1	1	1	1	1	1	1	1
	80	1	1	1	1	1	1	1	1	1	1	1

d = dead before termination of treatment  
 e = not recorded in error

**Table 12 Body weight – Individual values**

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Body weight and body weight gain (g)

Individual values - From arrival to Day 91

Males

GROUP	ANIMAL NO	ON ARRIVAL	DAY OF RE-ALLOCATION	BODY WT															
				DAY 1	DAY 7	DAY 14	DAY 21	DAY 28	DAY 35	DAY 42	DAY 49	DAY 56	DAY 63	DAY 70	DAY 77	DAY 84	DAY 91	DAY 1 TO 91 GAIN	
1	1	121	148	154	181	210	234	261	288	308	339	364	380	393	398	407	415	261	
	2	133	167	174	200	226	255	286	309	338	360	385	400	414	421	431	439	265	
	3	131	176	182	219	258	291	324	355	378	391	413	425	437	446	457	457	275	
	4	141	179	185	212	258	282	318	346	381	401	425	452	470	491	502	516	331	
	5	130	169	176	207	231	259	288	308	330	351	377	386	397	406	411	415	239	
	6	143	179	182	219	250	287	327	357	376	393	418	437	449	470	480	495	313	
	7	139	168	166	203	237	264	298	322	347	369	403	418	438	454	470	485	319	
	8	130	162	160	202	234	261	290	311	332	346	372	388	396	408	415	416	256	
	9	129	163	170	202	234	259	299	321	340	362	388	406	415	429	441	454	284	
	10	129	169	175	208	237	265	301	325	342	359	380	390	402	412	414	417	242	
2	21	d	134	174	181	218	252	282	263										
	22		139	177	185	213	245	270	249	318	344	371	402	416	432	443	444	454	269
	23		124	155	143	186	223	251	284	306	337	353	382	395	418	425	427	431	288
	24		130	160	148	192	219	250	274	293	322	336	358	370	386	391	405	408	260
	25		125	164	172	210	239	269	306	334	357	373	379	398	412	420	426	434	262
	26		126	159	167	202	231	265	294	318	340	354	372	388	399	416	426	428	261
	27		142	178	180	218	244	278	315	338	377	402	427	448	458	477	486	492	312
	28		134	170	175	207	239	274	304	334	362	383	405	419	432	446	458	468	293
	29		131	173	177	211	246	267	301	326	356	377	395	415	425	439	445	453	276
	30		137	165	169	200	234	257	293	316	352	377	411	425	445	463	469	481	312

d = dead before termination of treatment

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Body weight and body weight gain (g)

Individual values - From arrival to Day 91

Males

GROUP	ANIMAL NO	ON ARRIVAL	DAY OF RE-ALLOCATION	DAY OF															BODY WT	
				1	7	14	21	28	35	42	49	56	63	70	77	84	91	GAIN	DAY	
3	41	135	170	176	196	232	263	290	313	342	359	366	379	393	403	410	422	246		
	42	d 130	164	168	194	231	263													
	43	128	168	174	208	240	270	294	321	351	372	388	395	408	411	414	418	244		
	44	128	165	170	201	230	256	282	303	326	345	364	374	381	396	398	410	240		
	45	136	174	182	211	237	271	293	321	352	375	390	392	402	411	429	439	257		
	46	127	169	174	214	251	283	313	333	359	386	400	406	417	427	442	452	278		
	47	125	163	150	192	220	253	281	307	346	366	396	412	436	454	464	473	323		
	48	141	179	168	219	255	288	316	338	360	380	399	410	421	436	442	449	281		
	49	125	164	170	196	231	253	287	308	341	358	379	401	418	430	434	444	274		
	50	127	162	167	204	248	277	315	346	376	394	408	420	430	439	452	469	302		
4	61	128	167	172	206	235	264	289	308	335	362	384	401	417	430	441	447	275		
	62	132	171	177	209	243	273	302	325	356	381	397	415	423	437	439	450	273		
	63	135	162	167	197	225	254	283	306	335	359	372	388	401	408	411	418	251		
	64	141	177	182	219	253	274	304	329	342	354	357	367	376	395	402	411	229		
	65	125	156	164	183	216	253	287	320	348	368	395	415	431	456	465	482	318		
	66	142	175	183	204	230	256	282	309	334	352	371	384	399	415	428	436	253		
	67	130	169	169	202	228	265	308	341	370	385	394	410	421	440	452	456	287		
	68	141	178	174	211	234	267	295	323	352	375	391	402	413	424	434	436	262		
	69	139	169	176	206	229	267	308	335	372	389	410	427	444	456	470	479	303		
	70	131	159	166	206	227	268	305	328	352	377	393	396	415	420	427	438	272		

d = dead before termination of treatment

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Body weight and body weight gain (g)

Individual values - From arrival to Day 91

Females

GROUP	ANIMAL NO	ON ARRIVAL	DAY OF RE-ALLOCATION	DAY																BODY WT	
				1	7	14	21	28	35	42	49	56	63	70	77	84	91	GAIN	DAY		
1	11	128	150	155	168	198	197	226	244	250	250	262	262	276	285	289	287	132			
	12	121	147	151	160	175	190	210	223	229	230	243	252	250	258	262	272	121			
	13	125	151	148	162	180	191	209	224	231	235	244	250	247	252	260	263	115			
	14	126	151	157	162	193	182	216	247	228	245	252	268	263	257	269	285	128			
	15	135	154	154	166	194	210	212	210	224	233	239	246	246	240	254	255	101			
	16	120	146	146	159	176	191	197	215	219	230	226	237	240	245	256	249	103			
	17	139	163	166	181	210	232	234	249	274	288	259	270	269	273	281	281	115			
	18	133	151	156	167	186	200	212	226	233	234	226	245	248	254	253	259	103			
	19	135	162	166	181	193	218	228	239	249	258	261	264	265	271	274	276	110			
	20	114	138	144	167	182	201	220	221	237	244	255	255	257	264	267	271	127			
2	31	131	162	161	176	191	219	231	243	259	273	266	269	276	287	279	280	119			
	32	136	168	170	180	195	213	234	244	255	262	270	277	272	278	284	283	113			
	33	129	153	150	180	209	243	237	233	255	266	265	271	277	277	265	277	127			
	34	110	133	136	157	178	199	215	228	241	246	253	264	262	276	277	281	145			
	35	126	148	149	166	188	216	240	243	236	252	257	256	256	264	271	269	120			
	36	128	153	146	168	183	187	215	234	242	240	259	261	266	258	276	288	142			
	37	123	148	154	164	181	192	213	238	256	244	242	252	256	255	256	265	111			
	38	129	149	153	170	185	207	236	234	238	249	256	256	274	294	283	286	133			
	39	131	158	162	169	194	208	227	230	247	251	255	252	268	275	270	269	107			
	40	121	141	141	159	176	194	211	219	230	242	241	245	250	255	255	257	116			

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Body weight and body weight gain (g)

Individual values - From arrival to Day 91

Females

GROUP	ANIMAL NO	ON ARRIVAL	DAY OF RE-ALLOCATION	DAY 1	DAY 7	DAY 14	DAY 21	DAY 28	DAY 35	DAY 42	DAY 49	DAY 56	DAY 63	DAY 70	DAY 77	DAY 84	DAY 91	BODY WT		
																		GAIN	DAY 1 TO 91	
3	51	d	114	163	141	155														
	52		138	139	166	182	202	218	241	254	250	270	270	273	284	281	293	292	126	
	53		134	161	160	176	209	224	230	242	265	266	258	277	298	302	287	288	128	
	54		129	155	161	176	183	206	224	234	239	254	261	265	260	270	275	279	118	
	55	d	140	160	169	187														
	56		119	148	147	166	179	201	222	239	227	239	241	246	251	256	267	272	125	
	57		108	131	137	162	178	187	217	233	242	251	254	264	266	262	272	272	135	
	58		136	158	164	181	199	230	252	246	258	266	266	271	276	276	284	288	124	
	59		118	149	150	165	174	191	208	217	225	233	244	244	243	254	256	259	109	
	60		129	152	157	172	183	204	217	227	229	243	252	252	249	264	265	271	114	
	4	71		135	154	157	166	172	196	218	230	227	244	253	253	248	257	259	262	105
		72		132	157	164	171	195	212	222	239	250	253	252	260	265	268	271	276	112
73			139	157	161	180	183	213	231	227	228	245	251	255	253	261	266	266	105	
74			114	140	149	157	180	198	208	217	231	239	249	244	252	253	259	251	102	
75			117	148	155	175	191	209	230	248	259	259	256	275	287	276	280	274	119	
76			123	143	145	157	167	185	199	210	223	230	227	235	241	243	243	253	108	
77			130	155	152	175	200	202	232	250	247	264	282	276	278	267	280	290	138	
78			128	156	162	175	189	212	226	237	257	256	257	282	266	264	293	302	140	
79			128	149	150	167	176	196	215	214	223	236	239	242	252	251	244	257	107	
80			127	153	153	174	184	214	226	243	252	262	273	275	271	287	291	294	141	

d = dead before termination of treatment

**Table 13 Food consumption – Values per animal**

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Food consumption (g)

Values per animal - Day 1 - Day 91

Males

GROUP	CAGE NO	DAY	TOTAL												
		7	14	21	28	35	42	49	56	63	70	77	84	91	TO DAY 91
1	1	154.0	178.0	176.0	171.0	173.0	156.0	175.5	175.0	179.0	179.5	.	181.0	172.0	.
	2	166.0	192.0	190.0	190.5	186.5	.	183.0	172.5	194.0	194.0	194.5	196.0	193.5	.
	3	162.0	182.0	188.0	183.0	172.0	164.5	168.0	168.0	185.5	177.5	178.0	188.0	174.0	2290.5
	4	153.5	177.0	.	176.0	163.0	174.5	181.0	172.5	179.0	181.0	174.5	185.5	181.5	.
	5	150.0	174.0	174.0	181.0	162.5	155.0	165.0	158.0	167.5	173.5	172.0	162.5	160.5	2155.5
2	11	159.0	183.5	186.0	132.0	D	179.0	192.0	186.0	190.0	192.0	186.0	187.0	193.0	D
	12	170.0	190.0	197.0	184.5	167.0	180.5	185.5	180.0	182.5	187.0	180.0	196.5	175.5	2376.0
	13	168.5	184.0	187.0	182.5	169.0	156.5	161.0	127.5	182.0	173.0	167.5	164.0	170.0	2192.5
	14	168.5	191.0	207.5	197.5	192.5	188.5	211.0	183.0	203.5	192.5	199.0	203.5	192.0	2530.0
	15	156.0	179.5	188.0	197.5	197.0	171.5	191.0	184.5	198.0	191.5	192.5	200.5	195.0	2442.5
3	21	140.5	203.5	205.5	D	192.0	183.0	192.0	177.0	188.0	187.0	187.0	190.0	197.0	D
	22	170.0	202.0	204.5	182.0	178.0	156.5	170.5	168.5	177.0	175.5	173.0	173.5	172.0	2303.0
	23	159.0	186.5	188.5	179.0	169.5	170.5	175.5	168.0	179.5	171.5	181.5	190.0	187.5	2306.5
	24	165.5	199.5	200.5	182.5	181.5	170.0	182.0	177.0	191.0	189.0	191.5	194.0	188.0	2412.0
	25	171.5	206.5	220.5	205.0	186.5	188.5	190.5	182.0	204.0	208.5	203.5	213.5	214.5	2595.0
4	31	158.0	183.5	200.5	180.0	175.5	157.0	186.5	180.0	196.5	198.5	199.5	199.5	187.5	2402.5
	32	148.0	187.0	180.5	178.0	173.0	155.5	168.5	157.0	178.5	180.0	185.5	184.0	185.0	2260.5
	33	198.5	211.5	198.0	204.0	201.0	179.0	200.0	200.5	212.5	214.5	214.0	227.0	221.0	2681.5
	34	149.0	178.0	183.5	188.0	188.0	176.0	188.0	170.0	188.0	193.5	188.0	194.5	168.0	2352.5
	35	150.5	191.5	208.0	192.0	187.0	179.0	186.5	186.5	193.5	194.0	193.5	198.0	198.0	2458.0

. = not recorded in error

D = cage-mate dead before termination of treatment

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Food consumption (g)

Values per animal - Day 1 - Day 91

Females

GROUP	CAGE NO	DAY													TOTAL
		DAY 7	14	21	28	35	42	49	56	63	70	77	84	91	DAY 1 TO DAY 91
1	6	95.0	126.5	108.5	127.0	126.0	114.5	115.0	132.0	140.5	143.5	137.5	137.0	132.0	1635.0
	7	94.5	117.0	102.0	121.0	128.5	106.0	118.0	120.0	138.5	128.5	121.5	134.5	136.5	1566.5
	8	94.5	112.0	115.0	101.5	110.5	112.0	112.0	106.5	124.5	122.5	117.0	127.0	119.0	1474.0
	9	96.5	123.0	120.0	115.0	120.5	125.0	125.0	95.0	120.5	115.5	122.0	117.5	119.0	1514.5
	10	101.5	114.5	.	116.5	114.0	114.5	117.5	118.0	121.0	120.0	126.0	119.0	120.5	.
2	16	107.0	116.5	129.5	129.5	128.5	132.5	141.0	121.0	132.5	140.0	135.5	125.0	124.0	1662.5
	17	114.0	131.0	143.0	117.0	119.5	128.5	128.0	117.0	140.0	128.0	131.0	126.5	129.5	1653.0
	18	99.0	113.0	126.0	136.0	124.5	112.5	125.0	128.0	124.0	140.0	135.5	137.5	131.0	1632.0
	19	95.0	107.5	114.0	130.5	120.0	118.0	112.5	108.0	123.5	130.0	140.0	121.5	115.5	1536.0
	20	95.5	116.5	116.0	114.0	109.5	113.0	113.0	108.5	118.5	122.0	118.5	111.5	120.5	1477.0
3	26	96.5	D	125.0	137.0	132.0	124.0	132.0	134.0	129.0	146.0	134.0	139.0	128.0	D
	27	91.0	136.5	122.0	114.5	118.0	128.0	129.0	111.0	129.5	146.0	144.0	116.0	118.5	1604.0
	28	112.5	D	127.0	124.0	146.0	103.0	118.0	124.0	131.0	142.0	132.0	130.0	147.0	D
	29	115.0	128.5	140.5	134.0	119.0	115.5	130.0	121.0	134.5	128.5	133.0	135.5	130.5	1665.5
	30	99.5	114.0	119.5	121.0	119.0	113.0	126.0	120.0	127.5	132.0	137.5	123.5	126.0	1578.5
4	36	97.0	126.5	122.0	131.5	127.5	118.0	126.0	122.0	126.0	135.5	130.0	129.0	125.0	1616.0
	37	114.0	128.5	125.5	124.5	.	116.5	129.5	124.5	139.5	145.0	132.5	132.5	133.0	.
	38	112.5	129.5	130.0	125.5	125.0	126.5	121.0	115.0	135.0	139.0	124.5	127.0	129.5	1640.0
	39	117.5	133.0	113.5	133.5	136.0	124.5	126.0	129.5	149.5	126.0	124.0	139.0	152.5	1704.5
	40	102.5	122.0	111.5	129.5	117.5	116.5	126.5	130.0	135.0	145.0	141.0	129.0	131.0	1637.0

. = not recorded in error

D = cage-mate dead before termination of treatment

**Table 14 Water consumption – Values per animal**

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Water consumption (g)

Values per animal - Day 1 - Day 91

Males

GROUP	CAGE NO	DAY 1-3	DAY 3-7	DAY 7-10	DAY 10-14	DAY 14-17	DAY 17-21	DAY 21-24	DAY 24-28	DAY 28-31	DAY 31-35	DAY 35-38	DAY 38-42	DAY 42-45	DAY 45-49
1	1	44.5	75.5	57.0	80.5	62.5	83.0	53.5	77.5	58.0	81.0	60.0	92.0	61.0	82.5
	2	55.5	98.0	67.5	98.5	75.5	105.0	71.5	94.0	66.5	100.5	70.5	96.0	57.0	96.5
	3	48.5	87.0	60.5	80.5	67.5	78.5	63.5	87.5	64.5	.	57.0	83.0	54.0	76.5
	4	44.0	74.0	53.5	72.5	56.5	65.0	60.0	69.5	41.5	70.0	R	70.5	54.0	68.0
	5	55.5	94.0	65.0	95.5	66.0	87.5	70.5	90.0	60.0	82.0	61.0	74.0	59.5	74.0
2	11	112.0	97.5	70.0	93.0	75.5	80.5	63.5	7.5	94.5	D	49.0	114.0	74.0	108.0
	12	48.5	.	62.5	86.5	67.5	84.5	58.0	83.0	58.0	68.5	62.0	86.5	57.5	81.0
	13	64.5	101.5	59.0	99.0	83.0	90.5	73.0	19.5	75.0	89.5	67.5	105.0	63.0	75.5
	14	55.5	102.0	70.0	94.0	79.0	92.0	74.5	93.0	65.5	88.0	74.0	108.0	68.5	92.0
	15	49.0	85.0	58.0	88.5	66.0	79.5	65.5	84.0	62.5	84.5	60.0	117.0	58.5	82.5
3	21	99.0	60.0	59.5	65.0	R	91.0	63.0	D	75.0	90.0	77.0	96.0	73.0	94.0
	22	49.0	84.5	60.5	100.5	66.5	89.0	60.5	85.0	62.5	84.5	44.5	R	64.0	88.5
	23	54.0	.	.	93.0	67.5	89.5	59.5	87.5	61.5	84.0	69.5	108.5	65.0	89.0
	24	56.0	84.5	62.0	86.0	53.5	88.0	59.0	81.5	54.0	85.5	67.0	92.5	57.0	83.5
	25	58.5	93.5	55.5	90.5	74.5	99.0	69.5	93.0	.	87.5	71.5	112.0	66.5	89.0
4	31	50.0	95.0	54.0	82.5	68.0	93.0	67.5	89.0	57.5	84.5	70.0	85.0	63.0	95.0
	32	48.0	93.0	R	92.5	74.5	89.5	66.5	96.5	67.5	88.5	70.5	107.0	70.0	89.5
	33	52.0	88.0	71.0	80.0	83.5	96.5	81.0	100.0	81.5	101.0	77.5	129.0	66.0	105.5
	34	50.5	86.0	64.5	80.0	79.5	86.0	73.0	95.5	68.0	93.0	75.0	124.5	72.5	100.5
	35	54.5	91.0	73.0	94.0	81.5	89.0	78.0	R	74.0	97.0	75.5	128.0	71.0	103.5

D = cage-mate dead before termination of treatment  
 R = water bottle had been running  
 . = not recorded in error

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Water consumption (g)

Values per animal - Day 1 - Day 91

Males

GROUP	CAGE NO	DAY 49-52	DAY 52-56	DAY 56-59	DAY 59-63	DAY 63-66	DAY 66-70	DAY 70-73	DAY 73-77	DAY 77-80	DAY 80-84	DAY 84-87	DAY 87-91	TOTAL,
														DAY 91 TO
1	1	87.0	125.5	99.0	133.5	84.0	130.5	92.5	93.0	98.5	114.0	95.0	123.0	2244.0
	2	93.5	130.0	113.5	149.5	98.0	145.5	107.5	129.5	112.5	136.0	94.0	144.0	2606.0
	3	79.0	122.0	100.5	130.5	94.5	123.5	92.5	110.0	89.5	109.5	89.5	112.0	.
	4	81.5	115.5	87.0	119.5	78.5	115.0	80.0	107.5	85.0	115.0	93.0	111.5	.
	5	81.0	123.5	94.5	127.5	88.0	118.0	92.5	103.0	80.5	106.5	R	127.0	.
2	11	74.0	137.0	108.0	141.0	94.0	.	99.0	124.0	99.0	121.0	96.0	122.0	D
	12	73.0	115.0	95.5	124.0	80.0	118.0	75.5	107.5	88.5	111.0	84.0	111.0	.
	13	86.5	71.5	103.0	146.5	88.5	134.5	102.5	122.5	93.5	116.5	86.0	140.0	2357.0
	14	93.5	128.0	105.0	141.0	87.0	134.0	108.0	125.5	103.5	141.0	98.0	133.0	2553.5
	15	84.0	117.5	102.0	128.5	86.5	135.5	84.0	119.0	R	111.5	95.0	119.5	.
3	21	91.0	136.0	102.0	129.0	81.0	135.0	98.0	129.0	107.0	124.0	105.0	142.0	D
	22	89.0	124.5	98.0	132.0	88.5	132.5	92.0	120.5	96.5	114.0	91.5	118.0	.
	23	88.5	131.0	95.5	134.0	66.5	125.5	106.0	130.5	98.5	131.5	95.5	134.5	.
	24	75.5	121.0	89.5	133.0	87.0	127.5	87.0	116.5	93.5	124.0	86.5	119.0	2270.0
	25	85.5	133.0	101.5	150.0	96.5	150.0	102.0	130.5	111.5	141.5	104.5	142.0	.
4	31	92.5	133.5	109.0	154.0	97.5	149.5	112.5	142.0	109.5	144.5	107.5	155.0	2561.0
	32	93.5	133.5	111.0	157.0	95.0	142.5	109.0	137.0	108.5	141.0	108.5	139.5	.
	33	96.5	147.5	119.5	166.0	103.0	152.0	113.0	142.5	118.5	157.0	120.0	158.5	2806.5
	34	91.0	236.5	109.5	149.0	100.0	149.5	110.5	138.5	111.0	150.0	113.0	83.5	2690.5
	35	97.0	161.5	175.5	170.5	107.0	159.0	113.0	154.5	121.5	161.0	116.5	163.0	.

D = cage-mate dead before termination of treatment  
 R = water bottle had been running  
 . = not recorded in error

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Water consumption (g)

Values per animal - Day 1 - Day 91

Females

GROUP	CAGE NO	DAY 1-3	DAY 3-7	DAY 7-10	DAY 10-14	DAY 14-17	DAY 17-21	DAY 21-24	DAY 24-28	DAY 28-31	DAY 31-35	DAY 35-38	DAY 38-42	DAY 42-45	DAY 45-49
1	6	50.5	59.5	62.5	87.5	58.0	82.5	66.5	84.5	65.0	84.0	72.5	83.0	56.5	82.0
	7	44.5	64.0	54.5	73.5	47.5	80.0	57.0	74.0	58.5	82.0	48.0	75.0	57.0	76.0
	8	45.5	71.0	53.5	81.0	70.5	99.0	53.0	78.5	72.5	94.0	73.5	91.5	62.0	103.5
	9	35.5	57.5	43.0	64.0	51.0	77.0	45.5	62.0	52.0	64.5	48.0	71.0	49.5	65.5
	10	41.5	70.0	45.5	66.5	48.5	77.0	.	.	51.0	65.5	51.5	70.5	49.0	65.5
2	16	54.0	93.0	61.5	71.0	66.5	95.0	66.0	88.5	75.5	100.0	90.5	121.5	73.0	99.5
	17	58.0	94.0	59.5	100.5	82.0	103.5	61.5	86.0	60.0	87.0	91.0	106.0	67.5	R
	18	42.5	62.5	46.0	69.5	56.0	90.5	63.5	90.5	64.0	80.5	61.0	88.0	50.5	82.5
	19	36.5	61.0	40.0	73.5	44.5	80.0	53.0	72.5	50.5	68.5	59.5	83.5	47.0	62.0
	20	38.5	63.0	46.5	66.0	40.0	65.5	49.0	70.0	53.0	64.0	57.0	80.0	50.0	73.0
3	26	79.0	69.0	74.0	86.0	72.0	99.0	59.0	90.0	67.0	90.0	69.0	81.0	70.0	87.0
	27	45.5	65.0	45.0	72.5	65.0	94.0	52.0	75.5	54.5	72.5	67.5	92.5	50.0	74.0
	28	108.0	79.5	D	63.0	62.0	97.0	53.0	110.0	75.0	108.0	62.0	106.0	71.0	84.0
	29	43.0	83.0	52.5	71.5	52.0	87.0	60.0	76.0	R	76.0	60.5	89.5	56.5	84.0
	30	.	80.0	52.0	82.5	73.5	94.0	64.0	92.5	71.0	94.0	66.5	102.0	66.5	100.0
4	36	40.0	71.5	54.0	64.0	62.5	78.0	.	85.0	67.0	81.5	61.0	102.0	52.5	81.5
	37	47.5	86.5	55.0	76.0	64.5	84.0	59.0	R	68.5	86.0	69.5	96.0	67.5	89.0
	38	47.5	73.0	57.5	79.0	71.0	95.0	62.0	84.0	68.5	88.5	67.0	97.0	60.0	86.0
	39	39.5	75.5	60.5	75.0	55.0	77.5	63.0	87.0	67.5	83.5	70.0	122.0	57.0	72.0
	40	41.5	73.0	49.5	64.5	55.0	60.0	51.5	77.0	56.0	79.0	51.0	104.0	59.5	88.0

D = cage-mate dead before termination of treatment  
 R = water bottle had been running  
 . = not recorded in error

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Water consumption (g)

Values per animal - Day 1 - Day 91

Females

GROUP	CAGE NO	DAY 49-52	DAY 52-56	DAY 56-59	DAY 59-63	DAY 63-66	DAY 66-70	DAY 70-73	DAY 73-77	DAY 77-80	DAY 80-84	DAY 84-87	DAY 87-91	TOTAL,
														DAY 1 TO DAY 91
1	6	82.0	103.5	88.0	129.5	92.0	110.0	88.5	110.0	75.5	111.5	81.5	110.5	2177.0
	7	71.0	102.0	100.0	113.5	77.0	103.5	70.5	89.0	72.5	103.0	80.5	107.5	1981.5
	8	78.5	104.5	94.5	129.0	76.5	119.0	91.5	100.0	86.0	119.5	78.0	114.5	2240.5
	9	47.5	.	81.5	106.0	67.5	91.0	63.5	90.0	62.0	91.5	86.0	94.0	.
	10	66.5	86.5	67.5	100.5	64.5	92.0	71.5	91.0	63.5	91.0	66.0	83.0	.
2	16	87.5	R	98.0	140.0	84.5	121.0	R	117.5	77.0	120.5	83.5	124.5	.
	17	75.0	113.0	108.5	139.5	80.0	126.5	83.5	108.0	69.5	110.0	87.5	138.5	.
	18	80.0	115.0	82.0	125.0	82.5	132.0	100.5	110.0	97.5	109.5	81.0	120.5	2183.0
	19	50.5	84.0	76.5	100.0	63.5	104.0	78.5	96.5	76.5	96.5	75.0	82.5	1816.0
	20	62.5	86.0	77.5	101.0	61.5	91.0	66.0	89.5	52.5	93.5	69.0	90.5	1756.0
3	26	69.0	134.0	107.0	138.0	91.0	131.0	90.0	121.0	96.0	103.0	86.0	141.0	2399.0
	27	61.0	96.0	83.5	120.0	72.0	121.0	86.0	109.5	69.5	94.5	72.0	104.0	2014.5
	28	70.0	128.0	100.0	113.0	78.0	142.0	92.0	116.0	84.0	104.0	101.0	129.0	D
	29	68.0	104.0	85.5	122.5	71.5	R	73.0	103.5	74.0	104.0	79.5	105.0	.
	30	76.5	114.0	86.5	131.0	77.5	120.5	88.0	109.0	79.0	97.5	73.0	116.0	.
4	36	71.0	105.5	97.5	118.5	74.0	117.0	79.5	104.0	78.5	106.0	76.5	102.5	.
	37	80.0	120.5	98.5	131.0	89.5	132.5	95.0	113.0	82.0	99.0	89.5	121.5	.
	38	73.0	123.0	94.5	138.0	81.5	R	88.0	118.0	84.5	122.0	94.5	126.0	.
	39	77.0	118.0	100.5	131.0	77.0	106.0	74.5	106.5	83.0	133.5	83.0	124.0	2219.0
	40	R	124.0	95.0	130.5	85.0	126.0	91.5	120.5	86.5	122.5	87.0	117.0	.

D = cage-mate dead before termination of treatment

R = water bottle had been running

. = not recorded in error

### Table 15 Ophthalmoscopy – Individual findings

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Ophthalmoscopy

Group 1

Animal No/Sex	Before start of treatment	Before termination of treatment
1, male	No abnormal findings	No abnormal findings
2, male	No abnormal findings	No abnormal findings
3, male	Right eye: Slight central lenticular opacities Left eye: No abnormal findings	No abnormal findings
4, male	No abnormal findings	No abnormal findings
5, male	No abnormal findings	No abnormal findings
6, male	No abnormal findings	No abnormal findings
7, male	No abnormal findings	No abnormal findings
8, male	No abnormal findings	No abnormal findings
9, male	No abnormal findings	No abnormal findings
10, male	No abnormal findings	No abnormal findings
11, female	No abnormal findings	No abnormal findings
12, female	No abnormal findings	No abnormal findings
13, female	No abnormal findings	No abnormal findings
14, female	No abnormal findings	No abnormal findings
15, female	No abnormal findings	No abnormal findings
16, female	No abnormal findings	No abnormal findings
17, female	No abnormal findings	No abnormal findings
18, female	No abnormal findings	No abnormal findings
19, female	No abnormal findings	No abnormal findings
20, female	No abnormal findings	No abnormal findings

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Ophthalmoscopy

Group 2

Animal No/Sex	Before start of treatment	Before termination of treatment
21, male	No abnormal findings	
22, male	No abnormal findings	
23, male	No abnormal findings	
24, male	Right eye: Remnants of ocular membrane Left eye: No abnormal findings	
25, male	No abnormal findings	
26, male	No abnormal findings	
27, male	No abnormal findings	
28, male	Both eyes: Superficial corneal opacities	
29, male	No abnormal findings	
30, male	No abnormal findings	
31, female	No abnormal findings	
32, female	Both eyes: Superficial corneal opacities	
33, female	No abnormal findings	
34, female	No abnormal findings	
35, female	No abnormal findings	
36, female	No abnormal findings	
37, female	No abnormal findings	
38, female	No abnormal findings	
39, female	No abnormal findings	
40, female	No abnormal findings	

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Ophthalmoscopy

Group 3

Animal No/Sex	Before start of treatment	Before termination of treatment
41, male	No abnormal findings	
42, male	No abnormal findings	
43, male	No abnormal findings	
44, male	No abnormal findings	
45, male	No abnormal findings	
46, male	No abnormal findings	
47, male	No abnormal findings	
48, male	No abnormal findings	
49, male	No abnormal findings	
50, male	No abnormal findings	
51, female	No abnormal findings	
52, female	No abnormal findings	
53, female	No abnormal findings	
54, female	No abnormal findings	
55, female	No abnormal findings	
56, female	Right eye: Scare on cornea Left eye: No abnormal findings	
57, female	No abnormal findings	
58, female	No abnormal findings	
59, female	No abnormal findings	
60, female	No abnormal findings	

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Ophthalmoscopy

Group 4

<b>Animal No/Sex</b>	<b>Before start of treatment</b>	<b>Before termination of treatment</b>
61, male	No abnormal findings	No abnormal findings
62, male	Both eyes: Superficial corneal opacities	No abnormal findings
63, male	No abnormal findings	No abnormal findings
64, male	Right eye: No abnormal findings Left eye: Superficial corneal opacities	Both eyes: slight central lenticular opacities
65, male	No abnormal findings	No abnormal findings
66, male	No abnormal findings	No abnormal findings
67, male	No abnormal findings	No abnormal findings
68, male	No abnormal findings	No abnormal findings
69, male	No abnormal findings	No abnormal findings
70, male	No abnormal findings	No abnormal findings
71, female	No abnormal findings	No abnormal findings
72, female	No abnormal findings	No abnormal findings
73, female	No abnormal findings	No abnormal findings
74, female	No abnormal findings	No abnormal findings
75, female	No abnormal findings	No abnormal findings
76, female	No abnormal findings	No abnormal findings
77, female	No abnormal findings	No abnormal findings
78, female	No abnormal findings	No abnormal findings
79, female	No abnormal findings	No abnormal findings
80, female	No abnormal findings	No abnormal findings

**Table 16 Haematology – Individual values**

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Haematology

Individual values

Males

GROUP	ANIMAL NO	Hb	RBC	HT	MCV	MCH	MCHC	WBC	% NEUTRO	
									NEUTRO	NEUTRO
1	1	10.5	9.62	49	51	1.1	21.4	10.0	6	0.6
	2	10.3	9.28	48	52	1.1	21.4	7.7	12	0.9
	3	10.9	9.93	52	53	1.1	20.8	11.0	8	0.9
	4	10.6	9.71	49	51	1.1	21.5	12.3	8	1.0
	5	10.0	9.16	45	49	1.1	22.1	7.9	4	0.4
	6	8.0	9.32	47	51	0.9	16.9	8.0	5	0.4
	7	10.9	9.70	51	53	1.1	21.2	14.8	6	0.8
	8	10.1	9.71	49	51	1.0	20.6	8.1	5	0.4
	9	10.0	9.37	47	50	1.1	21.2	10.7	7	0.8
	10	10.6	10.53	51	48	1.0	20.8	12.3	5	0.6
2	21	d								
	22	10.6	9.69	50	51	1.1	21.3	12.1	5	0.6
	23	10.6	9.58	50	52	1.1	21.4	10.8	10	1.1
	24	10.2	9.56	48	51	1.1	21.0	11.7	9	1.0
	25	10.2	9.59	48	50	1.1	21.2	12.2	8	1.0
	26	10.6	9.67	49	51	1.1	21.5	10.4	5	0.6
	27	10.6	9.62	49	51	1.1	21.5	11.9	7	0.8
	28	10.0	8.96	47	53	1.1	21.1	9.1	3	0.3
	29	10.4	9.39	48	51	1.1	21.8	8.8	4	0.3
	30	10.3	9.57	48	50	1.1	21.5	9.7	4	0.4

Abbreviations and units are explained in subsection 'Clinical Pathology'

d = dead before termination of treatment

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Haematology

Individual values

Males

GROUP	ANIMAL NO	Hb	RBC	HT	MCV	MCH	MCHC	WBC	%	
									NEUTRO	NEUTRO
3	41	10.0	10.01	48	48	1.0	21.0	5.8	10	0.6
	42	d								
	43	10.6	9.66	50	51	1.1	21.4	9.2	12	1.1
	44	10.8	9.52	51	53	1.1	21.3	13.6	6	0.8
	45	10.2	9.45	48	51	1.1	21.2	10.5	9	1.0
	46	10.0	9.30	48	51	1.1	21.0	7.5	3	0.2
	47	10.4	8.89	48	54	1.2	21.7	8.9	5	0.4
	48	10.3	9.45	48	51	1.1	21.4	10.3	8	0.8
	49	10.9	9.27	50	54	1.2	21.7	11.3	10	1.1
	50	10.7	9.87	50	51	1.1	21.4	11.9	8	1.0
4	61	10.1	9.79	49	50	1.0	20.5	12.7	14	1.8
	62	11.1	9.84	52	53	1.1	21.4	14.5	9	1.3
	63	10.3	9.56	48	51	1.1	21.4	7.8	9	0.7
	64	10.5	9.32	50	54	1.1	20.9	13.4	11	1.5
	65	10.4	9.15	49	53	1.1	21.3	18.3	7	1.2
	66	10.6	9.98	50	50	1.1	21.2	10.8	7	0.7
	67	10.4	9.21	48	52	1.1	21.5	10.4	5	0.5
	68	10.8	9.79	50	51	1.1	21.6	8.5	5	0.4
	69	10.6	9.06	50	55	1.2	21.4	14.6	13	2.0
	70	10.9	9.51	52	54	1.2	21.1	16.8	5	0.9

Abbreviations and units are explained in subsection 'Clinical Pathology'

d = dead before termination of treatment

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Haematology

Individual values

Males

GROUP	ANIMAL NO	% LYMPHO	LYMPHO	% EOS	EOS	% BASO	BASO	% MONO	MONO	Plt	APTT	Pt	Fib
1	1	91	9.1	2	0.2	0	0.0	1	0.1	832	18.2	17.1	3.09
	2	85	6.5	2	0.2	0	0.0	1	0.1	109	14.4	16.2	3.19
	3	89	9.8	2	0.2	0	0.0	1	0.1	698	22.0	16.4	3.50
	4	88	10.7	3	0.4	0	0.0	1	0.1	227	19.2	17.0	3.52
	5	93	7.3	2	0.1	0	0.0	1	0.1	666	18.7	17.7	2.87
	6	93	7.4	1	0.1	0	0.0	1	0.1	1041	16.2	15.3	2.98
	7	92	13.5	1	0.2	0	0.0	2	0.2	725	18.5	15.6	3.10
	8	91	7.3	2	0.2	0	0.0	2	0.2	293	25.7	15.0	2.96
	9	90	9.6	1	0.2	0	0.0	1	0.1	750	17.0	14.7	3.02
	10	87	10.8	2	0.2	0	0.0	6	0.7	640	27.2	17.7	1.99
2	21	d											
	22	92	11.2	2	0.2	0	0.0	1	0.1	821	15.4	17.6	2.97
	23	87	9.4	1	0.2	0	0.0	1	0.1	731	14.9	16.5	3.18
	24	89	10.4	2	0.2	0	0.0	1	0.1	837	16.5	16.6	3.04
	25	87	10.6	3	0.4	0	0.0	1	0.1	804	17.2	16.2	3.12
	26	90	9.4	2	0.2	0	0.0	3	0.3	709	12.2	16.5	3.63
	27	88	10.5	2	0.3	0	0.0	3	0.3	740	21.2	15.0	2.89
	28	94	8.6	2	0.2	0	0.0	2	0.1	871	16.5	16.5	3.50
	29	93	8.1	1	0.1	0	0.0	2	0.2	748	19.7	16.6	2.97
	30	92	8.9	2	0.2	0	0.0	2	0.2	766	15.7	15.2	3.20

Abbreviations and units are explained in subsection 'Clinical Pathology'

d = dead before termination of treatment

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Haematology

Individual values

Males

GROUP	ANIMAL NO	% LYMPHO	LYMPHO	% EOS	EOS	% BASO	BASO	% MONO	MONO	Plt	APTT	Pt	Fib
3	41	88	5.1	2	0.1	0	0.0	0	0.0	776	16.7	16.6	2.88
	42	d											
	43	84	7.7	2	0.2	1	0.1	1	0.1	513	16.2	16.8	3.03
	44	91	12.4	1	0.2	1	0.2	1	0.1	562	15.2	16.8	3.05
	45	87	9.1	2	0.2	0	0.0	2	0.2	653	13.4	17.3	3.04
	46	91	6.8	1	0.1	0	0.0	5	0.4	740	14.2	15.8	3.26
	47	89	8.0	3	0.2	0	0.0	3	0.3	814	18.7	15.4	3.37
	48	85	8.8	2	0.2	0	0.0	4	0.4	806	13.2	16.4	3.46
	49	84	9.5	2	0.2	0	0.0	5	0.5	804	17.7	15.9	3.66
	50	86	10.3	2	0.2	0	0.0	4	0.4	684	21.7	14.9	3.10
4	61	82	10.4	2	0.3	0	0.0	2	0.2	710	22.0	16.6	3.92
	62	88	12.8	2	0.3	0	0.0	1	0.1	591	17.5	16.8	2.97
	63	88	6.8	2	0.1	1	0.1	1	0.1	701	14.7	16.2	3.17
	64	86	11.5	2	0.2	0	0.0	1	0.1	647	15.2	16.5	2.98
	65	91	16.6	2	0.3	0	0.1	1	0.2	826	16.7	16.8	3.37
	66	90	9.7	1	0.2	0	0.0	2	0.2	796	17.0	15.2	3.18
	67	90	9.3	2	0.2	0	0.0	3	0.4	739	16.5	15.3	3.18
	68	90	7.7	1	0.1	0	0.0	3	0.3	730	15.4	14.3	2.69
	69	81	11.8	3	0.4	0	0.1	3	0.4	622	22.0	15.9	2.66
	70	90	15.2	2	0.3	0	0.0	3	0.5	677	18.2	15.6	3.05

Abbreviations and units are explained in subsection 'Clinical Pathology'

d = dead before termination of treatment

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Haematology

Individual values

Females

GROUP	ANIMAL NO	Hb	RBC	HT	MCV	MCH	MCHC	WBC	% NEUTRO	
									NEUTRO	NEUTRO
1	11	9.7	8.92	47	53	1.1	20.7	12.1	7	0.9
	12	10.2	8.99	47	53	1.1	21.5	11.8	7	0.8
	13	9.6	8.63	45	52	1.1	21.6	10.1	5	0.5
	14	9.7	8.51	45	53	1.1	21.6	7.4	9	0.6
	15	9.7	9.06	46	51	1.1	21.2	8.6	5	0.5
	16	10.2	9.05	47	52	1.1	21.6	9.7	5	0.5
	17	9.7	8.77	45	52	1.1	21.5	10.1	3	0.3
	18	9.7	8.44	44	52	1.2	21.9	7.1	6	0.4
	19	9.4	8.53	44	52	1.1	21.4	5.3	6	0.3
	20	10.1	8.76	47	54	1.2	21.4	6.9	5	0.3
2	31	9.5	8.54	44	51	1.1	21.6	5.4	5	0.3
	32	9.8	8.75	45	51	1.1	21.8	10.9	8	0.9
	33	9.6	8.49	45	52	1.1	21.5	4.5	7	0.3
	34	9.6	8.24	46	55	1.2	21.1	13.5	5	0.7
	35	9.2	7.96	42	53	1.2	21.8	6.0	9	0.6
	36	9.5	8.33	45	54	1.1	21.0	E	5	0.5
	37	9.4	8.43	44	52	1.1	21.6	10.6	7	0.8
	38	9.9	8.79	45	52	1.1	21.7	8.9	5	0.4
	39	9.6	8.67	45	52	1.1	21.3	5.1	4	0.2
	40	9.7	8.72	46	52	1.1	21.3	8.3	5	0.4

Abbreviations and units are explained in subsection 'Clinical Pathology'

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Haematology

Individual values

Females

GROUP	ANIMAL NO		Hb	RBC	HT	MCV	MCH	MCHC	WBC	% NEUTRO	
										NEUTRO	NEUTRO
3	51	d									
	52		9.9	8.90	46	51	1.1	21.6	6.4	8	0.5
	53		10.2	8.62	48	56	1.2	21.2	7.8	8	0.6
	54		10.0	8.88	47	53	1.1	21.5	7.4	7	0.5
	55	d									
	56		9.6	8.17	44	54	1.2	21.7	7.1	5	0.3
	57		10.6	8.97	49	55	1.2	21.6	7.0	7	0.5
	58		10.3	8.95	47	53	1.2	21.8	6.9	7	0.5
	59		9.4	8.49	43	51	1.1	21.8	7.4	5	0.4
	60		9.8	8.92	46	52	1.1	21.3	E	5	0.5
4	71		9.4	8.64	45	52	1.1	20.9	7.6	11	0.8
	72		9.8	8.74	46	53	1.1	21.3	6.9	7	0.5
	73		9.8	8.67	46	53	1.1	21.4	7.5	9	0.6
	74		9.9	9.16	46	51	1.1	21.4	8.5	8	0.6
	75		10.2	8.47	48	57	1.2	21.1	10.4	8	0.9
	76		9.4	8.32	45	54	1.1	21.0	5.9	9	0.5
	77		9.7	8.46	44	52	1.2	21.9	6.3	7	0.4
	78		9.4	8.68	44	51	1.1	21.4	10.4	2	0.3
	79		9.3	8.09	43	53	1.2	21.6	4.2	6	0.3
	80		10.0	8.70	47	54	1.2	21.4	6.1	5	0.3

Abbreviations and units are explained in subsection 'Clinical Pathology'

d = dead before termination of treatment, no sample

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Haematology

Individual values

Females

GROUP	ANIMAL NO	% LYMPHO	LYMPHO	% EOS	EOS	% BASO	BASO	% MONO	MONO	Plt	APTT	Pt	Fib
1	11	90	10.9	2	0.2	0	0.0	1	0.1	847	17.7	17.9	2.44
	12	91	10.7	1	0.2	0	0.0	1	0.1	760	15.4	17.4	2.54
	13	94	9.5	1	0.1	0	0.0	1	0.1	796	15.2	17.1	2.49
	14	88	6.5	2	0.2	1	0.1	1	0.1	766	13.7	16.6	2.50
	15	92	7.9	2	0.2	1	0.0	0	0.0	845	14.2	17.1	2.78
	16	91	8.8	2	0.2	1	0.1	1	0.1	711	15.2	15.3	2.61
	17	93	9.4	2	0.2	0	0.0	2	0.2	818	15.7	17.0	2.76
	18	91	6.4	2	0.2	0	0.0	1	0.1	850	17.2	15.4	2.50
	19	89	4.7	2	0.1	0	0.0	2	0.1	920	13.2	16.8	2.69
	20	91	6.3	3	0.2	0	0.0	2	0.1	723	15.2	16.6	1.75
2	31	92	5.0	2	0.1	0	0.0	1	0.1	845	16.5	18.3	2.49
	32	89	9.8	2	0.2	0	0.0	0	0.0	826	E	17.7	2.85
	33	90	4.1	2	0.1	1	0.0	0	0.0	750	E	17.0	2.59
	34	92	12.4	2	0.2	1	0.1	1	0.1	815	14.9	16.5	2.50
	35	86	5.2	3	0.2	1	0.0	1	0.0	941	14.4	16.2	2.37
	36	92	7.9	2	0.1	0	0.0	1	0.1	647	14.4	15.6	2.72
	37	90	9.5	2	0.2	1	0.1	1	0.1	823	16.2	15.2	2.21
	38	92	8.2	2	0.2	1	0.1	1	0.1	773	18.5	15.8	2.56
	39	95	4.8	1	0.1	0	0.0	1	0.0	799	14.7	16.2	2.63
	40	92	7.6	1	0.1	0	0.0	1	0.1	767	27.0	16.8	2.30

Abbreviations and units are explained in subsection 'Clinical Pathology'

E = measuring error

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Haematology

Individual values

Females

GROUP	ANIMAL NO	% LYMPHO	% LYMPHO	% EOS	% EOS	% BASO	% BASO	% MONO	% MONO	Plt	APTT	Pt	Fib
3	51	d											
	52	88	5.7	3	0.2	1	0.0	1	0.1	865	11.2	16.2	1.84
	53	88	6.9	3	0.3	0	0.0	1	0.1	797	13.9	16.2	1.90
	54	90	6.7	2	0.2	0	0.0	1	0.0	805	17.7	16.2	2.30
	55	d									36.7	C	1.39
	56	91	6.5	3	0.2	0	0.0	2	0.1	729	16.7	15.2	2.47
	57	89	6.3	3	0.2	0	0.0	1	0.1	799	16.5	15.4	2.47
	58	90	6.2	2	0.1	0	0.0	1	0.1	852	12.4	16.2	2.81
	59	92	6.8	2	0.1	0	0.0	1	0.1	787	14.9	16.8	2.61
	60	92	6.8	2	0.2	0	0.0	1	0.1	883	16.2	15.9	2.51
4	71	85	6.4	3	0.2	1	0.1	1	0.1	978	15.7	17.1	2.65
	72	91	6.2	2	0.1	1	0.0	0	0.0	812	14.7	17.4	2.69
	73	88	6.7	3	0.2	0	0.0	0	0.0	774	16.2	15.8	2.40
	74	91	7.7	1	0.1	0	0.0	1	0.1	663	15.9	16.6	2.70
	75	88	9.2	2	0.2	1	0.1	1	0.1	692	15.4	16.5	2.46
	76	84	5.0	6	0.4	0	0.0	0	0.0	1024	14.4	15.3	2.66
	77	89	5.6	4	0.2	0	0.0	1	0.1	749	15.9	15.9	2.63
	78	96	9.9	1	0.1	0	0.0	1	0.1	830	16.2	15.4	2.54
	79	88	3.6	6	0.2	0	0.0	1	0.0	803	15.4	16.1	2.63
	80	92	5.7	3	0.2	0	0.0	1	0.0	713	14.7	15.4	2.98

Abbreviations and units are explained in subsection 'Clinical Pathology'

d = dead before termination of treatment, no sample

C = coagulated

**Table 17 Clinical chemistry – Individual values**

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Clinical chemistry

Individual values

Males

GROUP	ANIMAL NO	ALAT	ASAT	ALKPH	BILI	GGT	CHOL	TRIG	UREA	CREAT	GLUC
1	1	1.21	1.41	2.09	<LOD	<LOD	2.3	1.27	5.85	31	6.1
	2	1.18	2.22	1.76	<LOD	<LOD	2.4	1.12	7.23	34	10.0
	3	1.08	1.43	2.80	1.4	<LOD	2.1	1.13	5.92	29	6.0
	4	1.44	2.62	3.28	<LOD	<LOD	3.2	2.41	6.56	28	6.1
	5	0.85	1.34	2.57	<LOD	<LOD	2.1	1.39	6.42	25	6.1
	6	1.22	1.70	2.35	<LOD	<LOD	2.4	2.16	8.20	30	6.3
	7	1.25	1.34	3.11	<LOD	<LOD	2.5	1.22	8.29	31	7.1
	8	1.34	1.87	2.54	<LOD	<LOD	1.9	1.40	6.11	25	7.4
	9	0.86	1.16	2.68	<LOD	<LOD	2.7	2.70	7.21	29	6.9
	10	1.15	2.28	2.19	<LOD	<LOD	2.1	1.41	7.60	29	6.1
2	21	d									
	22	1.07	1.88	2.69	<LOD	<LOD	2.4	1.57	7.12	25	5.1
	23	0.97	1.37	2.56	<LOD	<LOD	2.7	1.44	6.64	26	6.6
	24	1.07	1.66	2.45	<LOD	<LOD	2.5	1.60	7.80	29	5.9
	25	1.30	2.08	2.96	<LOD	<LOD	2.2	2.03	7.53	25	6.1
	26	0.89	1.40	2.72	<LOD	<LOD	2.0	1.68	6.97	28	6.2
	27	1.24	1.77	3.01	<LOD	<LOD	2.5	1.99	8.26	30	6.2
	28	1.09	1.82	2.80	1.4	<LOD	2.2	1.95	6.64	27	6.5
	29	1.51	2.22	2.69	<LOD	<LOD	2.3	1.07	7.37	28	6.0
	30	1.04	1.30	2.53	<LOD	<LOD	2.3	1.83	6.69	24	7.3

Abbreviations and units are explained in subsection 'Clinical Pathology'

Limit of detection for BILI is 1.3

Limit of detection for GGT is 0.04

d = dead before termination of treatment

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Clinical chemistry

Individual values

Males

GROUP	ANIMAL NO	ALAT	ASAT	ALKPH	BILI	GGT	CHOL	TRIG	UREA	CREAT	GLUC	
3	41	1.15	1.40	3.61	<LOD	<LOD	2.1	1.08	9.53	25	6.0	
	42	d										
	43	0.90	2.03	3.37	1.4	<LOD	2.0	1.04	7.59	27	5.9	
	44	0.97	2.05	3.17	1.4	<LOD	1.9	0.98	6.85	30	6.3	
	45	0.92	1.60	2.68	<LOD	<LOD	1.9	0.95	6.87	24	6.5	
	46	0.86	1.36	2.37	<LOD	<LOD	1.8	1.65	6.82	30	6.6	
	47	1.63	1.95	2.84	<LOD	<LOD	2.1	1.74	6.41	27	6.3	
	48	⊠	0.90	2.35	2.09	<LOD	<LOD	1.9	1.68	6.94	25	6.8
	49	0.93	1.66	2.29	<LOD	<LOD	2.5	1.26	7.51	27	6.5	
	50	1.14	1.40	3.13	<LOD	<LOD	2.8	2.15	7.63	28	5.9	
4	61	0.74	2.27	2.61	<LOD	<LOD	2.4	1.73	6.37	26	5.5	
	62	0.91	2.07	3.37	<LOD	<LOD	1.8	1.17	7.87	28	6.4	
	63	1.16	1.69	3.40	<LOD	<LOD	2.2	1.34	7.81	26	5.8	
	64	0.83	1.90	2.87	<LOD	<LOD	2.1	1.37	7.07	23	6.1	
	65	0.92	1.50	3.65	<LOD	<LOD	2.0	1.28	7.63	31	6.6	
	66	0.91	1.82	3.43	<LOD	<LOD	2.1	1.29	6.69	25	5.7	
	67	1.54	1.75	2.84	<LOD	<LOD	2.0	0.95	8.23	29	6.0	
	68	1.05	1.78	3.11	<LOD	<LOD	2.7	1.01	7.34	32	6.6	
	69	1.07	1.21	3.02	1.4	<LOD	2.2	2.32	8.04	30	5.9	
	70	1.00	2.04	2.73	<LOD	<LOD	2.2	2.02	8.65	27	6.3	

Abbreviations and units are explained in subsection 'Clinical Pathology'

Limit of detection for BILI is 1.3

Limit of detection for GGT is 0.04

d = dead before termination of treatment

⊠ = haemolysis in blood sample - results excluded from statistical analysis

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Clinical chemistry

Individual values

Males

GROUP	ANIMAL NO	Na	K	Ca	Mg	P	Cl	PROTEIN	ALB	GLOBULIN	ALB/G Ratio
1	1	145.7	6.18	2.74	0.97	2.58	102.6	71.7	46	25.7	1.79
	2	144.6	5.74	2.67	0.93	2.34	102.0	65.2	41	24.2	1.69
	3	145.3	5.02	2.74	0.99	2.48	100.6	75.6	49	26.6	1.84
	4	145.8	6.85	2.95	1.02	2.41	98.9	84.3	51	33.3	1.53
	5	143.7	6.15	2.76	0.90	2.12	102.8	68.3	46	22.3	2.06
	6	139.9	6.53	2.77	0.96	2.30	99.7	71.2	44	27.2	1.62
	7	142.3	5.04	2.86	0.88	2.41	101.0	73.9	48	25.9	1.85
	8	141.5	5.79	2.74	0.88	2.31	101.5	69.9	45	24.9	1.81
	9	142.8	5.67	2.97	0.91	2.45	99.1	70.9	45	25.9	1.74
	10	142.8	6.34	2.79	0.95	2.46	99.6	74.4	47	27.4	1.72
2	21	d									
	22	146.0	6.09	2.65	0.95	2.27	101.1	72.9	46	26.9	1.71
	23	144.8	6.35	2.74	0.87	2.32	101.6	67.7	43	24.7	1.74
	24	142.6	7.58	2.76	1.01	2.57	100.4	69.4	44	25.4	1.73
	25	141.6	7.26	2.79	1.00	2.41	100.1	70.0	46	24.0	1.92
	26	142.7	5.42	2.74	0.89	2.16	101.3	67.2	44	23.2	1.90
	27	142.6	5.59	2.79	0.94	2.21	100.1	72.9	47	25.9	1.81
	28	144.8	6.02	2.90	0.98	2.50	100.4	72.7	46	26.7	1.72
	29	145.0	4.94	2.75	0.87	2.04	101.6	69.9	43	26.9	1.60
	30	143.5	5.44	2.85	0.84	2.45	100.6	75.3	48	27.3	1.76

Abbreviations and units are explained in subsection 'Clinical Pathology'

d = dead before termination of treatment

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Clinical chemistry

Individual values

Males

GROUP	ANIMAL NO	Na	K	Ca	Mg	P	Cl	PROTEIN	ALB	GLOBULIN	ALB/G Ratio	
3	41	147.5	5.65	2.71	0.81	1.93	103.8	68.3	45	23.3	1.93	
	42	d										
	43	143.6	5.56	2.75	0.90	2.09	102.1	69.9	47	22.9	2.05	
	44	141.2	7.62	2.67	0.95	2.01	100.7	68.7	47	21.7	2.17	
	45	143.7	6.45	2.78	0.91	2.29	102.2	68.1	45	23.1	1.95	
	46	143.6	5.43	2.78	0.91	2.37	100.4	67.5	43	24.5	1.76	
	47	142.4	5.44	2.79	0.82	2.24	99.7	71.3	45	26.3	1.71	
	48	⌘	139.1	7.70	2.73	0.91	2.31	100.7	69.1	43	26.1	1.65
	49	142.7	5.19	2.82	0.94	2.44	99.7	75.6	48	27.6	1.74	
	50	144.7	5.63	2.84	0.96	2.15	99.4	74.5	48	26.5	1.81	
4	61	143.4	6.57	2.73	0.95	2.13	101.4	71.8	45	26.8	1.68	
	62	143.3	6.39	2.69	0.94	2.18	101.3	71.9	47	24.9	1.89	
	63	142.9	6.73	2.64	0.96	2.17	99.7	71.2	45	26.2	1.72	
	64	140.5	7.47	2.59	0.95	2.19	99.1	68.6	45	23.6	1.91	
	65	145.0	7.00	2.85	0.96	2.26	101.1	71.9	45	26.9	1.67	
	66	140.3	6.92	2.71	0.86	2.15	98.7	71.5	46	25.5	1.80	
	67	142.5	6.36	2.69	0.90	1.97	101.2	70.0	46	24.0	1.92	
	68	140.2	5.89	2.65	1.01	1.80	98.1	69.3	44	25.3	1.74	
	69	142.3	4.59	2.99	0.98	2.59	98.4	77.3	48	29.3	1.64	
	70	140.7	7.59	2.75	0.96	2.46	99.8	72.2	45	27.2	1.65	

Abbreviations and units are explained in subsection 'Clinical Pathology'

d = dead before termination of treatment

⌘ = haemolysis in blood sample - results excluded from statistical analysis

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Clinical chemistry

Individual values

Females

GROUP	ANIMAL NO	ALAT	ASAT	ALKPH	BILI	GGT	CHOL	TRIG	UREA	CREAT	GLUC
1	11	0.91	1.61	2.57	1.7	<LOD	2.1	0.47	6.65	25	7.6
	12	0.92	1.96	1.69	1.6	<LOD	2.1	0.56	5.78	30	8.6
	13	0.92	1.58	1.56	<LOD	<LOD	2.2	0.92	5.84	28	6.2
	14	1.22	1.46	1.92	1.4	<LOD	2.7	0.72	7.78	29	6.4
	15	0.89	1.90	1.64	<LOD	<LOD	2.2	0.40	5.88	30	5.4
	16	0.97	1.91	1.67	<LOD	<LOD	3.2	0.76	5.88	28	6.6
	17	0.91	1.82	2.07	<LOD	<LOD	2.2	0.88	5.42	28	7.0
	18	0.92	2.13	1.83	<LOD	<LOD	2.4	0.31	6.79	35	6.3
	19	0.94	1.75	2.12	<LOD	<LOD	2.3	0.44	7.37	27	6.0
	20	0.80	1.73	2.37	<LOD	<LOD	2.0	0.35	7.35	35	6.9
2	31	0.79	1.18	1.49	<LOD	<LOD	3.3	0.37	6.61	33	6.7
	32	0.75	1.25	2.01	<LOD	<LOD	2.8	0.94	6.56	28	5.7
	33	0.84	1.63	1.28	1.7	<LOD	2.8	0.44	6.92	34	5.9
	34	0.73	1.18	1.58	1.4	<LOD	2.5	0.97	7.76	28	5.7
	35	0.98	1.50	1.94	1.6	<LOD	3.1	0.64	7.68	28	6.4
	36	0.90	1.95	2.08	<LOD	<LOD	2.2	0.49	7.70	31	7.9
	37	0.68	1.55	2.40	1.7	<LOD	2.2	0.65	6.08	33	6.1
	38	0.65	2.19	1.92	1.7	<LOD	2.1	0.50	6.20	29	6.7
	39	0.75	1.27	1.39	<LOD	<LOD	2.9	0.51	6.17	32	6.3
	40	0.73	1.58	1.85	<LOD	<LOD	2.8	1.02	9.10	29	5.7

Abbreviations and units are explained in subsection 'Clinical Pathology'

Limit of detection for BILI is 1.3

Limit of detection for GGT is 0.04

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Clinical chemistry

Individual values

Females

GROUP	ANIMAL NO	ALAT	ASAT	ALKPH	BILI	GGT	CHOL	TRIG	UREA	CREAT	GLUC
3	51	d									
	52	0.79	1.46	2.23	1.6	<LOD	2.1	0.55	6.35	28	6.0
	53	0.87	1.90	2.06	1.5	<LOD	2.1	0.38	7.34	32	6.4
	54	0.74	1.69	1.90	1.6	<LOD	2.8	0.45	6.01	28	7.2
	55	d									
	56	0.65	1.41	1.28	<LOD	<LOD	2.3	0.41	5.55	25	6.0
	57	0.87	1.74	2.62	<LOD	<LOD	2.5	1.33	7.33	32	6.2
	58	0.72	1.69	2.33	<LOD	<LOD	2.1	0.27	5.93	32	6.1
	59	0.69	1.11	1.62	<LOD	<LOD	2.6	0.46	5.69	29	6.0
	60	0.84	1.45	1.76	<LOD	<LOD	2.3	0.83	6.41	28	5.7
4	71	0.79	1.58	2.09	1.4	<LOD	2.3	0.78	6.51	29	5.2
	72	0.84	1.71	2.23	1.5	<LOD	2.5	0.58	6.65	28	7.0
	73	0.79	1.38	1.97	<LOD	<LOD	2.8	0.80	8.97	34	5.5
	74	0.66	1.55	1.74	3.2	<LOD	2.6	0.35	6.19	30	7.4
	75	0.70	1.41	1.35	1.4	<LOD	2.5	0.80	8.53	31	6.3
	76	0.57	1.24	2.05	<LOD	<LOD	2.8	0.28	6.44	33	5.8
	77	0.85	1.66	2.35	<LOD	<LOD	1.6	0.86	8.43	30	7.4
	78	0.72	1.58	2.72	1.6	<LOD	2.7	0.88	5.02	29	6.9
	79	0.74	1.43	1.81	<LOD	<LOD	2.0	0.39	7.40	34	5.5
	80	0.89	1.43	2.02	<LOD	<LOD	3.0	0.55	8.42	32	6.4

Abbreviations and units are explained in subsection 'Clinical Pathology'

Limit of detection for BILI is 1.3

Limit of detection for GGT is 0.04

d = dead before termination of treatment

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Clinical chemistry

Individual values

Females

GROUP	ANIMAL NO	Na	K	Ca	Mg	P	Cl	PROTEIN	ALB	GLOBULIN	ALB/G Ratio
1	11	143.0	4.84	2.80	0.89	2.21	102.7	73.5	50	23.5	2.13
	12	141.3	5.90	2.60	1.10	2.18	100.9	65.1	45	20.1	2.24
	13	144.1	6.41	2.66	0.96	2.10	101.7	64.4	45	19.4	2.32
	14	141.8	5.84	2.80	0.91	1.93	100.9	71.5	50	21.5	2.33
	15	146.4	5.97	2.81	1.11	2.24	106.2	70.7	48	22.7	2.11
	16	139.5	6.52	2.76	1.04	2.26	98.8	72.5	49	23.5	2.09
	17	141.8	6.69	2.73	0.96	2.31	101.9	67.7	45	22.7	1.98
	18	143.3	6.98	2.79	1.10	1.43	103.9	75.2	51	24.2	2.11
	19	140.3	6.71	2.67	0.91	1.41	101.7	70.4	49	21.4	2.29
	20	142.4	4.83	2.56	1.02	1.24	104.1	68.7	50	18.7	2.67
2	31	141.1	5.70	2.70	0.99	1.55	100.9	71.0	48	23.0	2.09
	32	142.8	5.84	2.68	0.94	1.85	101.0	71.8	48	23.8	2.02
	33	145.6	4.69	2.66	1.01	1.96	104.2	68.3	48	20.3	2.36
	34	143.4	6.43	2.94	1.06	2.11	100.7	76.7	55	21.7	2.53
	35	145.0	6.15	2.73	1.00	2.38	102.5	65.7	46	19.7	2.34
	36	141.2	5.05	2.71	0.85	1.44	104.1	67.2	46	21.2	2.17
	37	140.7	5.07	2.81	0.96	2.23	101.2	69.7	50	19.7	2.54
	38	140.8	5.98	2.77	0.98	2.02	101.6	70.6	47	23.6	1.99
	39	140.5	6.51	2.74	1.00	1.98	100.4	68.0	47	21.0	2.24
	40	141.7	5.77	2.59	0.99	1.50	102.8	66.5	45	21.5	2.09

Abbreviations and units are explained in subsection 'Clinical Pathology'

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Clinical chemistry

Individual values

Females

GROUP	ANIMAL NO	Na	K	Ca	Mg	P	Cl	PROTEIN	ALB	GLOBULIN	ALB/G Ratio
3	51	d									
	52	142.9	6.36	2.80	1.03	2.00	101.3	74.3	53	21.3	2.49
	53	145.2	5.79	2.61	1.07	1.67	104.2	69.1	48	21.1	2.27
	54	142.3	6.62	2.71	0.99	2.18	100.9	69.5	49	20.5	2.39
	55	d									
	56	138.8	6.73	2.77	0.96	2.36	100.1	69.0	46	23.0	2.00
	57	141.3	5.37	2.66	0.98	1.52	102.8	74.1	52	22.1	2.35
	58	141.5	5.84	2.59	0.90	1.26	103.3	69.5	47	22.5	2.09
	59	141.5	5.61	2.72	0.88	1.69	101.8	66.6	48	18.6	2.58
	60	141.5	6.26	2.78	0.97	1.73	101.0	73.4	52	21.4	2.43
4	71	142.9	6.75	2.80	1.06	2.30	101.6	72.1	51	21.1	2.42
	72	142.5	5.90	2.84	0.94	2.20	102.1	71.1	49	22.1	2.22
	73	142.2	6.79	2.83	1.14	1.77	101.0	78.5	56	22.5	2.49
	74	141.3	6.06	2.86	0.98	2.05	101.3	76.2	50	26.2	1.91
	75	143.6	6.29	2.78	1.14	2.49	103.5	69.1	49	20.1	2.44
	76	139.4	6.18	2.67	0.91	1.28	101.6	69.5	48	21.5	2.23
	77	139.1	5.53	2.58	0.95	1.73	101.3	64.5	45	19.5	2.31
	78	139.9	5.67	2.81	0.93	2.21	100.0	71.6	48	23.6	2.03
	79	143.0	7.03	2.74	0.94	1.53	104.6	70.4	48	22.4	2.14
	80	140.0	6.46	2.69	0.93	1.40	101.0	70.8	48	22.8	2.11

Abbreviations and units are explained in subsection 'Clinical Pathology'

d = dead before termination of treatment

**Table 18 Organ weight – Individual values**

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Organ weight (mg)

Individual values

Males

GROUP	ANIMAL NO	ADRENALS	BRAIN	EPIDI-DYMIDES	HEART	KIDNEYS	LIVER	SPLEEN	TESTES	THYMUS	
1	1	42	2212	1188	1336	2744	14035	737	3661	441	
	2	42	2353	1430	1701	3031	15766	808	3573	516	
	3	48	2192	1470	1688	2866	16645	946	3790	428	
	4	60	2129	1228	1566	3319	21139	989	3441	323	
	5	53	2154	1362	1448	2758	14027	826	3608	323	
	6	71	2132	1515	1683	2911	18920	831	3805	407	
	7	60	2227	1570	1721	2929	18242	919	4105	789	
	8	56	2125	1230	1464	3094	15117	738	3476	518	
	9	76	2252	1367	1345	2774	16888	873	3760	520	
	10	62	2229	1338	1548	2833	15152	896	3961	351	
2	21	d	48	2133	802	1266	2181	10862	430	3223	633
	22	60	2151	1465	1619	3418	17301	858	3908	378	
	23	55	2339	1383	1584	3076	14934	854	3648	444	
	24	49	2136	1302	1399	2555	13863	726	3469	332	
	25	46	2062	1199	1367	3056	16676	802	3615	243	
	26	44	2175	1459	1434	2596	13644	796	3413	653	
	27	67	2274	1538	1629	2942	18497	991	3914	589	
	28	69	2284	1472	1607	3241	16934	958	4063	500	
	29	62	2221	1162	1484	3030	15855	884	3667	443	
	30	68	2286	1302	1469	3614	17832	996	3806	364	

d = dead before termination of treatment - results excluded from statistical analysis

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Organ weight (mg)

Individual values

Males

GROUP	ANIMAL NO	ADRENALS	BRAIN	EPIDI-DYMIDES	HEART	KIDNEYS	LIVER	SPLEEN	TESTES	THYMUS	
3	41	51	2180	1346	1452	2984	14324	690	4196	345	
	42	d	40	2089	812	1132	2433	13184	781	2988	738
	43	52	2352	1421	1491	2657	13494	783	3685	360	
	44	67	2118	1424	1366	2454	13285	708	3809	492	
	45	45	2321	1273	1472	3171	14298	921	3730	609	
	46	77	2222	1412	1467	2906	14647	822	3843	534	
	47	58	2163	1359	1564	3082	16830	789	3802	425	
	48	68	2226	1457	1768	3185	14058	822	3978	437	
	49	69	2069	1392	1640	3157	15945	893	3744	414	
	50	70	2208	1476	1580	2968	16935	992	3840	502	
4	61	76	2182	1522	1491	3203	15893	799	3803	365	
	62	53	2266	1327	1426	2679	14375	849	3866	669	
	63	52	2377	1200	1461	2798	13983	715	3508	281	
	64	47	2130	1335	1421	2755	14525	1089	3972	537	
	65	44	2228	1111	1546	3031	16999	771	3596	541	
	66	56	2172	1391	1429	3308	14835	929	3754	522	
	67	49	2110	1351	1692	2881	16307	921	3542	498	
	68	52	2059	1239	1410	2601	16183	812	3498	402	
	69	45	2302	1479	1816	3569	18298	1202	3652	675	
	70	60	2183	1312	1484	2788	14596	925	4181	497	

d = dead before termination of treatment - results excluded from statistical analysis

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Relative (% of body wt) organ weight

Individual values

Males

GROUP	ANIMAL NO	BODY WT, g	ADRENALS	BRAIN	EPIDI - DYMIDES	HEART	KIDNEYS	LIVER	SPLEEN	TESTES	THYMUS
1	1	415	0.0101	0.533	0.286	0.322	0.661	3.38	0.178	0.882	0.106
	2	439	0.0096	0.536	0.326	0.387	0.690	3.59	0.184	0.814	0.118
	3	457	0.0105	0.480	0.322	0.369	0.627	3.64	0.207	0.829	0.094
	4	516	0.0116	0.413	0.238	0.303	0.643	4.10	0.192	0.667	0.063
	5	415	0.0128	0.519	0.328	0.349	0.665	3.38	0.199	0.869	0.078
	6	492	0.0144	0.433	0.308	0.342	0.592	3.85	0.169	0.773	0.083
	7	484	0.0124	0.460	0.324	0.356	0.605	3.77	0.190	0.848	0.163
	8	419	0.0134	0.507	0.294	0.349	0.738	3.61	0.176	0.830	0.124
	9	453	0.0168	0.497	0.302	0.297	0.612	3.73	0.193	0.830	0.115
	10	418	0.0148	0.533	0.320	0.370	0.678	3.62	0.214	0.948	0.084
2	21	d 308	0.0156	0.693	0.260	0.411	0.708	3.53	0.140	1.046	0.206
	22	454	0.0132	0.474	0.323	0.357	0.753	3.81	0.189	0.861	0.083
	23	431	0.0128	0.543	0.321	0.368	0.714	3.46	0.198	0.846	0.103
	24	408	0.0120	0.524	0.319	0.343	0.626	3.40	0.178	0.850	0.081
	25	434	0.0106	0.475	0.276	0.315	0.704	3.84	0.185	0.833	0.056
	26	430	0.0102	0.506	0.339	0.333	0.604	3.17	0.185	0.794	0.152
	27	499	0.0134	0.456	0.308	0.326	0.590	3.71	0.199	0.784	0.118
	28	471	0.0146	0.485	0.313	0.341	0.688	3.60	0.203	0.863	0.106
	29	456	0.0136	0.487	0.255	0.325	0.664	3.48	0.194	0.804	0.097
	30	484	0.0140	0.472	0.269	0.304	0.747	3.68	0.206	0.786	0.075

d = dead before termination of treatment - results excluded from statistical analysis

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Relative (% of body wt) organ weight

Individual values

Males

GROUP	ANIMAL NO	BODY WT, g	ADRENALS	BRAIN	EPIDI - DYMIDES	HEART	KIDNEYS	LIVER	SPLEEN	TESTES	THYMUS
3	41	422	0.0121	0.517	0.319	0.344	0.707	3.39	0.164	0.994	0.082
	42	d 278	0.0144	0.751	0.292	0.407	0.875	4.74	0.281	1.075	0.265
	43	418	0.0124	0.563	0.340	0.357	0.636	3.23	0.187	0.882	0.086
	44	410	0.0163	0.517	0.347	0.333	0.599	3.24	0.173	0.929	0.120
	45	439	0.0103	0.529	0.290	0.335	0.722	3.26	0.210	0.850	0.139
	46	447	0.0172	0.497	0.316	0.328	0.650	3.28	0.184	0.860	0.119
	47	477	0.0122	0.453	0.285	0.328	0.646	3.53	0.165	0.797	0.089
	48	448	0.0152	0.497	0.325	0.395	0.711	3.14	0.183	0.888	0.098
	49	443	0.0156	0.467	0.314	0.370	0.713	3.60	0.202	0.845	0.093
	50	466	0.0150	0.474	0.317	0.339	0.637	3.63	0.213	0.824	0.108
4	61	447	0.0170	0.488	0.340	0.334	0.717	3.56	0.179	0.851	0.082
	62	450	0.0118	0.504	0.295	0.317	0.595	3.19	0.189	0.859	0.149
	63	418	0.0124	0.569	0.287	0.350	0.669	3.35	0.171	0.839	0.067
	64	411	0.0114	0.518	0.325	0.346	0.670	3.53	0.265	0.966	0.131
	65	482	0.0091	0.462	0.230	0.321	0.629	3.53	0.160	0.746	0.112
	66	431	0.0130	0.504	0.323	0.332	0.768	3.44	0.216	0.871	0.121
	67	456	0.0107	0.463	0.296	0.371	0.632	3.58	0.202	0.777	0.109
	68	442	0.0118	0.466	0.280	0.319	0.588	3.66	0.184	0.791	0.091
	69	485	0.0093	0.475	0.305	0.374	0.736	3.77	0.248	0.753	0.139
	70	437	0.0137	0.500	0.300	0.340	0.638	3.34	0.212	0.957	0.114

d = dead before termination of treatment - results excluded from statistical analysis

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Relative (% of brain wt) organ weight

Individual values

Males

GROUP	ANIMAL NO	ADRENALS	EPIDI-DYMIDES	HEART	KIDNEYS	LIVER	SPLEEN	TESTES	THYMUS	
1	1	1.90	53.7	60.4	124.1	634.5	33.3	165.5	19.9	
	2	1.78	60.8	72.3	128.8	670.0	34.3	151.8	21.9	
	3	2.19	67.1	77.0	130.7	759.4	43.2	172.9	19.5	
	4	2.82	57.7	73.6	155.9	992.9	46.5	161.6	15.2	
	5	2.46	63.2	67.2	128.0	651.2	38.3	167.5	15.0	
	6	3.33	71.1	78.9	136.5	887.4	39.0	178.5	19.1	
	7	2.69	70.5	77.3	131.5	819.1	41.3	184.3	35.4	
	8	2.64	57.9	68.9	145.6	711.4	34.7	163.6	24.4	
	9	3.37	60.7	59.7	123.2	749.9	38.8	167.0	23.1	
	10	2.78	60.0	69.4	127.1	679.8	40.2	177.7	15.7	
2	21	d	2.25	37.6	59.4	102.3	509.2	20.2	151.1	29.7
	22		2.79	68.1	75.3	158.9	804.3	39.9	181.7	17.6
	23		2.35	59.1	67.7	131.5	638.5	36.5	156.0	19.0
	24		2.29	61.0	65.5	119.6	649.0	34.0	162.4	15.5
	25		2.23	58.1	66.3	148.2	808.7	38.9	175.3	11.8
	26		2.02	67.1	65.9	119.4	627.3	36.6	156.9	30.0
	27		2.95	67.6	71.6	129.4	813.4	43.6	172.1	25.9
	28		3.02	64.4	70.4	141.9	741.4	41.9	177.9	21.9
	29		2.79	52.3	66.8	136.4	713.9	39.8	165.1	19.9
	30		2.97	57.0	64.3	158.1	780.1	43.6	166.5	15.9

d = dead before termination of treatment - results excluded from statistical analysis

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Relative (% of brain wt) organ weight

Individual values

Males

GROUP	ANIMAL NO	ADRENALS	EPIDI- DYMIDES	HEART	KIDNEYS	LIVER	SPLEEN	TESTES	THYMUS	
3	41	2.34	61.7	66.6	136.9	657.1	31.7	192.5	15.8	
	42	d	1.91	38.9	54.2	116.5	631.1	37.4	143.0	35.3
	43		2.21	60.4	63.4	113.0	573.7	33.3	156.7	15.3
	44		3.16	67.2	64.5	115.9	627.2	33.4	179.8	23.2
	45		1.94	54.8	63.4	136.6	616.0	39.7	160.7	26.2
	46		3.47	63.5	66.0	130.8	659.2	37.0	173.0	24.0
	47		2.68	62.8	72.3	142.5	778.1	36.5	175.8	19.6
	48		3.05	65.5	79.4	143.1	631.5	36.9	178.7	19.6
	49		3.33	67.3	79.3	152.6	770.7	43.2	181.0	20.0
	50		3.17	66.8	71.6	134.4	767.0	44.9	173.9	22.7
4	61	3.48	69.8	68.3	146.8	728.4	36.6	174.3	16.7	
	62	2.34	58.6	62.9	118.2	634.4	37.5	170.6	29.5	
	63	2.19	50.5	61.5	117.7	588.3	30.1	147.6	11.8	
	64	2.21	62.7	66.7	129.3	681.9	51.1	186.5	25.2	
	65	1.97	49.9	69.4	136.0	763.0	34.6	161.4	24.3	
	66	2.58	64.0	65.8	152.3	683.0	42.8	172.8	24.0	
	67	2.32	64.0	80.2	136.5	772.8	43.6	167.9	23.6	
	68	2.53	60.2	68.5	126.3	786.0	39.4	169.9	19.5	
	69	1.95	64.2	78.9	155.0	794.9	52.2	158.6	29.3	
	70	2.75	60.1	68.0	127.7	668.6	42.4	191.5	22.8	

d = dead before termination of treatment - results excluded from statistical analysis

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Organ weight (mg)

Individual values

Females

GROUP	ANIMAL NO	ADRENALS	BRAIN	HEART	KIDNEYS	LIVER	OVARIES	SPLEEN	THYMUS	UTERUS
1	11	71	2193	1165	1904	9459	141	648	338	592
	12	76	2157	1008	1846	8827	78	585	331	779
	13	87	2014	1064	1873	8927	126	613	374	724
	14	57	2104	948	1856	9813	120	567	349	420
	15	56	2099	956	1812	7501	102	556	204	1113
	16	71	2085	1015	1728	9249	96	699	235	548
	17	78	1900	1084	1901	9659	115	706	349	527
	18	82	2176	1022	1798	8898	111	525	263	658
	19	77	2028	929	1913	9568	113	659	370	1198
	20	56	1888	1084	1736	8958	155	626	423	1437
2	31	73	2029	1004	1882	10093	91	615	301	748
	32	73	2182	1026	2006	9240	81	627	243	753
	33	61	1997	1249	1669	9202	100	464	214	930
	34	68	2098	1122	2080	12675	112	846	298	512
	35	66	2069	917	1826	9451	75	576	232	535
	36	81	2121	1106	2024	10200	125	644	408	897
	37	64	1972	1066	1784	7867	122	570	214	619
	38	75	2089	1062	2025	8889	125	576	311	721
	39	85	2148	911	1857	8580	114	578	315	739
	40	65	2018	1033	1793	8695	118	658	328	603

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Organ weight (mg)

Individual values

Females

GROUP	ANIMAL NO		ADRENALS	BRAIN	HEART	KIDNEYS	LIVER	OVARIES	SPLEEN	THYMUS	UTERUS
3	51	d	39	1886	718	1302	7625	88	479	661	427
	52		72	2104	960	1918	9507	87	598	248	1473
	53		76	2054	1177	1653	8821	123	656	332	509
	54		71	2142	1035	1971	9346	102	536	261	730
	55	d	54	1931	935	1488	8577	70	501	583	611
	56		71	2163	1016	1820	8923	132	547	265	623
	57		64	2008	1108	1854	8316	95	673	364	1688
	58		76	2056	1122	1818	8579	137	722	242	1057
	59		71	2153	958	1828	8272	114	517	289	656
	60		70	2046	1079	2013	9048	116	630	246	654
4	71		86	2002	969	1759	8864	102	570	264	447
	72		66	2105	1040	1846	8779	111	539	278	531
	73		60	1977	1279	1750	8680	144	562	245	579
	74		76	2179	991	1744	7297	89	522	311	809
	75		65	2018	970	1838	8761	82	709	289	621
	76		77	2023	1004	1666	7767	103	536	295	716
	77		72	2120	1031	2106	9364	146	678	416	459
	78		70	2022	1144	2276	9934	99	625	461	767
	79		70	1888	1044	1639	8350	147	489	260	807
	80		76	2125	1108	1858	9655	98	688	313	806

d = dead before termination of treatment - results excluded from statistical analysis

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Relative (% of body wt) organ weight

Individual values

Females

GROUP	ANIMAL NO	BODY WT, g	ADRENALS	BRAIN	HEART	KIDNEYS	LIVER	OVARIES	SPLEEN	THYMUS	UTERUS
1	11	287	0.0247	0.764	0.406	0.663	3.30	0.0491	0.226	0.118	0.206
	12	272	0.0279	0.793	0.371	0.679	3.25	0.0287	0.215	0.122	0.286
	13	263	0.0331	0.766	0.405	0.712	3.39	0.0479	0.233	0.142	0.275
	14	285	0.0200	0.738	0.333	0.651	3.44	0.0421	0.199	0.122	0.147
	15	255	0.0220	0.823	0.375	0.711	2.94	0.0400	0.218	0.080	0.436
	16	252	0.0282	0.827	0.403	0.686	3.67	0.0381	0.277	0.093	0.217
	17	283	0.0276	0.671	0.383	0.672	3.41	0.0406	0.249	0.123	0.186
	18	253	0.0324	0.860	0.404	0.711	3.52	0.0439	0.208	0.104	0.260
	19	275	0.0280	0.737	0.338	0.696	3.48	0.0411	0.240	0.135	0.436
	20	272	0.0206	0.694	0.399	0.638	3.29	0.0570	0.230	0.156	0.528
2	31	280	0.0261	0.725	0.359	0.672	3.60	0.0325	0.220	0.108	0.267
	32	283	0.0258	0.771	0.363	0.709	3.27	0.0286	0.222	0.086	0.266
	33	277	0.0220	0.721	0.451	0.603	3.32	0.0361	0.168	0.077	0.336
	34	281	0.0242	0.747	0.399	0.740	4.51	0.0399	0.301	0.106	0.182
	35	269	0.0245	0.769	0.341	0.679	3.51	0.0279	0.214	0.086	0.199
	36	284	0.0285	0.747	0.389	0.713	3.59	0.0440	0.227	0.144	0.316
	37	263	0.0243	0.750	0.405	0.678	2.99	0.0464	0.217	0.081	0.235
	38	278	0.0270	0.751	0.382	0.728	3.20	0.0450	0.207	0.112	0.259
	39	274	0.0310	0.784	0.332	0.678	3.13	0.0416	0.211	0.115	0.270
	40	257	0.0253	0.785	0.402	0.698	3.38	0.0459	0.256	0.128	0.235

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Relative (% of body wt) organ weight

Individual values

Females

GROUP	ANIMAL		BODY	ADRENALS	BRAIN	HEART	KIDNEYS	LIVER	OVARIES	SPLEEN	THYMUS	UTERUS
	NO		WT, g									
3	51	d	155	0.0252	1.217	0.463	0.840	4.92	0.0568	0.309	0.426	0.275
	52		292	0.0247	0.721	0.329	0.657	3.26	0.0298	0.205	0.085	0.504
	53		288	0.0264	0.713	0.409	0.574	3.06	0.0427	0.228	0.115	0.177
	54		279	0.0254	0.768	0.371	0.706	3.35	0.0366	0.192	0.094	0.262
	55	d	186	0.0290	1.038	0.503	0.800	4.61	0.0376	0.269	0.313	0.328
	56		265	0.0268	0.816	0.383	0.687	3.37	0.0498	0.206	0.100	0.235
	57		270	0.0237	0.744	0.410	0.687	3.08	0.0352	0.249	0.135	0.625
	58		281	0.0270	0.732	0.399	0.647	3.05	0.0488	0.257	0.086	0.376
	59		254	0.0280	0.848	0.377	0.720	3.26	0.0449	0.204	0.114	0.258
	60		271	0.0258	0.755	0.398	0.743	3.34	0.0428	0.232	0.091	0.241
4	71		262	0.0328	0.764	0.370	0.671	3.38	0.0389	0.218	0.101	0.171
	72		276	0.0239	0.763	0.377	0.669	3.18	0.0402	0.195	0.101	0.192
	73		266	0.0226	0.743	0.481	0.658	3.26	0.0541	0.211	0.092	0.218
	74		251	0.0303	0.868	0.395	0.695	2.91	0.0355	0.208	0.124	0.322
	75		274	0.0237	0.736	0.354	0.671	3.20	0.0299	0.259	0.105	0.227
	76		245	0.0314	0.826	0.410	0.680	3.17	0.0420	0.219	0.120	0.292
	77		294	0.0245	0.721	0.351	0.716	3.19	0.0497	0.231	0.141	0.156
	78		296	0.0236	0.683	0.386	0.769	3.36	0.0334	0.211	0.156	0.259
	79		253	0.0277	0.746	0.413	0.648	3.30	0.0581	0.193	0.103	0.319
	80		294	0.0259	0.723	0.377	0.632	3.28	0.0333	0.234	0.106	0.274

d = dead before termination of treatment - results excluded from statistical analysis

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Relative (% of brain wt) organ weight

Individual values

Females

GROUP	ANIMAL NO	ADRENALS	HEART	KIDNEYS	LIVER	OVARIES	SPLEEN	THYMUS	UTERUS
1	11	3.24	53.1	86.8	431.3	6.43	29.5	15.4	27.0
	12	3.52	46.7	85.6	409.2	3.62	27.1	15.3	36.1
	13	4.32	52.8	93.0	443.2	6.26	30.4	18.6	35.9
	14	2.71	45.1	88.2	466.4	5.70	26.9	16.6	20.0
	15	2.67	45.5	86.3	357.4	4.86	26.5	9.7	53.0
	16	3.41	48.7	82.9	443.6	4.60	33.5	11.3	26.3
	17	4.11	57.1	100.1	508.4	6.05	37.2	18.4	27.7
	18	3.77	47.0	82.6	408.9	5.10	24.1	12.1	30.2
	19	3.80	45.8	94.3	471.8	5.57	32.5	18.2	59.1
	20	2.97	57.4	91.9	474.5	8.21	33.2	22.4	76.1
2	31	3.60	49.5	92.8	497.4	4.48	30.3	14.8	36.9
	32	3.35	47.0	91.9	423.5	3.71	28.7	11.1	34.5
	33	3.05	62.5	83.6	460.8	5.01	23.2	10.7	46.6
	34	3.24	53.5	99.1	604.1	5.34	40.3	14.2	24.4
	35	3.19	44.3	88.3	456.8	3.62	27.8	11.2	25.9
	36	3.82	52.1	95.4	480.9	5.89	30.4	19.2	42.3
	37	3.25	54.1	90.5	398.9	6.19	28.9	10.9	31.4
	38	3.59	50.8	96.9	425.5	5.98	27.6	14.9	34.5
	39	3.96	42.4	86.5	399.4	5.31	26.9	14.7	34.4
	40	3.22	51.2	88.9	430.9	5.85	32.6	16.3	29.9

Serine endopeptidase (PPA 26797)

A 13-Week Oral (Gavage) Toxicity Study in Rats

Relative (% of brain wt) organ weight

Individual values

Females

GROUP	ANIMAL NO		ADRENALS	HEART	KIDNEYS	LIVER	OVARIES	SPLEEN	THYMUS	UTERUS
3	51	d	2.07	38.1	69.0	404.3	4.67	25.4	35.0	22.6
	52		3.42	45.6	91.2	451.9	4.13	28.4	11.8	70.0
	53		3.70	57.3	80.5	429.5	5.99	31.9	16.2	24.8
	54		3.31	48.3	92.0	436.3	4.76	25.0	12.2	34.1
	55	d	2.80	48.4	77.1	444.2	3.63	25.9	30.2	31.6
	56		3.28	47.0	84.1	412.5	6.10	25.3	12.3	28.8
	57		3.19	55.2	92.3	414.1	4.73	33.5	18.1	84.1
	58		3.70	54.6	88.4	417.3	6.66	35.1	11.8	51.4
	59		3.30	44.5	84.9	384.2	5.29	24.0	13.4	30.5
	60		3.42	52.7	98.4	442.2	5.67	30.8	12.0	32.0
4	71		4.30	48.4	87.9	442.8	5.09	28.5	13.2	22.3
	72		3.14	49.4	87.7	417.1	5.27	25.6	13.2	25.2
	73		3.03	64.7	88.5	439.0	7.28	28.4	12.4	29.3
	74		3.49	45.5	80.0	334.9	4.08	24.0	14.3	37.1
	75		3.22	48.1	91.1	434.1	4.06	35.1	14.3	30.8
	76		3.81	49.6	82.4	383.9	5.09	26.5	14.6	35.4
	77		3.40	48.6	99.3	441.7	6.89	32.0	19.6	21.7
	78		3.46	56.6	112.6	491.3	4.90	30.9	22.8	37.9
	79		3.71	55.3	86.8	442.3	7.79	25.9	13.8	42.7
	80		3.58	52.1	87.4	454.4	4.61	32.4	14.7	37.9

d = dead before termination of treatment - results excluded from statistical analysis

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**Appendix I      Pathology report  
(81 pages, excl. this cover page)**

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PROJECT : 20076029

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TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
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-----

EXPLANATION OF CODES AND SYMBOLS  
-----

CODES AND SYMBOLS USED AT ANIMAL LEVEL:  
-----

M = Male animal  
F = Female animal  
K0 = Terminal sacrifice group  
+ = Intercurrent death/sacrificed moribund  
+1 = Found dead  
+2 = Sacrificed moribund

CODES AND SYMBOLS USED AT ORGAN LEVEL:  
-----

G = Gross observation checked off histologically  
\* = Comment in text of individual animal data  
0 = Tissue not present for histologic examination  
' = Histologic examination not required  
+ = Organ examined, findings present  
- = Organ examined, no pathologic findings noted (AOFT only)

CODES AND SYMBOLS USED AT FINDING LEVEL:  
-----

GRADE 1 = Minimal / very few / very small  
GRADE 2 = Slight / few / small  
GRADE 3 = Moderate / moderate number / moderate size  
P = Finding present, severity not scored  
( = Finding unilateral in paired organs  
\* = Comment in text of individual animal data

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NUMBER OF ANIMALS WITH NECROPSY FINDINGS BY ORGAN/GROUP/SEX  
 STATUS AT NECROPSY: K0, INCL. DEATHS

ORGAN/FINDING	DOSE GROUP: 01		02		03		04	
	SEX:		M	F	M	F	M	F
	ANIM.EXAM.:		10	10	10	10	10	10
LUNG	:							
- discoloration, red.	:		-	-	-	-	2	-
ESOPHAGUS	:							
- perforation.	:		-	-	-	-	1	-
THYMUS	:							
- discoloration: red.	:		-	-	-	-	1	-
- hemorrhage.	:		-	1	-	-	-	1
BODY CAVITIES	:							
- containing blood/blood clots.	:		-	-	-	-	2	-
- contains reddish-watery fluid, edema.	:		-	-	-	-	1	-
- edema.	:		-	-	-	-	1	-































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TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)  
DOSE GROUP : 03, 165.1 mg TOS/kg

ANIMAL NUMBER :

	51	52	53	54	55	56	57	58	59	60
	FK0+	FK0	FK0	FK0	FK0+	FK0	FK0	FK0	FK0	FK0
GENERAL OBSERVATIONS	*									
BRAIN	-				-					
SPINAL CORD, CERVIC.	-				-					
SPINAL CORD, THORAC.	-				-					
SPINAL CORD, LUMBAR	-				-					
SCIATIC NERVE, RIGHT	-				-					
HEART	-				-					
AORTA	-				-					
TRACHEA	-				-					
LUNG	+G				+G					
- Alveolar hemorrhage	3.				3.					
ESOPHAGUS	-				-G					
STOMACH NONGLANDULAR	-				-					
STOMACH GLANDULAR	-				-					
DUODENUM	-				-					
JEJUNUM	-				-					
ILEUM	-				-					
CECUM	-				-					
COLON	-				-					
RECTUM	-				-					

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 SPONSOR : Novozymes A/S PathData@System V6.2a2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)  
 DOSE GROUP : 03, 165.1 mg TOS/kg

ANIMAL NUMBER :

	51	52	53	54	55	56	57	58	59	60
	FK0+	FK0	FK0	FK0	FK0+	FK0	FK0	FK0	FK0	FK0
LIVER	-	'	'	'	+	'	'	'	'	'
- Vacuolation hepatoc.	.				2.					
PANCREAS	-	'	'	'	-	'	'	'	'	'
KIDNEYS	-	'	'	'	+	'	'	'	'	'
- Tubular bas/dil foc.	.				( 1.					
URINARY BLADDER	-	'	'	'	-	'	'	'	'	'
OVARIES	-	'	'	'	-	'	'	'	'	'
UTERUS	-*	'	'	'	-*	'	'	'	'	'
CERVIX	-	'	'	'	-	'	'	'	'	'
VAGINA	-	'	'	'	-	'	'	'	'	'
PITUITARY GLAND	-	'	'	'	-	'	'	'	'	'
THYROID GLAND	-	'	'	'	-	'	'	'	'	'
PARATHYROID GLANDS	0				-					
ADRENAL GLANDS	-	'	'	'	-	'	'	'	'	'
SPLEEN	-	'	'	'	-	'	'	'	'	'
THYMUS	+G	'	'	'	+	'	'	'	'	'
- Hemorrhage, focal	1.				1.					
MESENT. LYMPH NODE	-	'	'	'	-	'	'	'	'	'
MANDIBULAR LN RIGHT	-	'	'	'	-	'	'	'	'	'
PAROTID GLAND, RIGHT	-	'	'	'	-	'	'	'	'	'
SUBLING. GLAND, RIGHT	-	'	'	'	-	'	'	'	'	'

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TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)  
DOSE GROUP : 03, 165.1 mg TOS/kg

ANIMAL NUMBER :

	51	52	53	54	55	56	57	58	59	60
	FK0+	FK0	FK0	FK0	FK0+	FK0	FK0	FK0	FK0	FK0
SUBMANDIB. GLD. RIGHT	-	'	'	'	-	'	'	'	'	'
MAMMARY GLAND	-	'	'	'	-	'	'	'	'	'
SKIN/SUBCUTIS	-	'	'	'	-	'	'	'	'	'
SKELETAL MUSCLE	-	'	'	'	-	'	'	'	'	'
EYES	-	'	'	'	-	'	'	'	'	'
OPTIC NERVES	-	'	'	'	-	'	'	'	'	'
BODY CAVITIES	'G	'	'	'	'G	'	'	'	'	'
STERNUM	-	'	'	'	-	'	'	'	'	'



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 SPONSOR : Novozymes A/S PathData@System V6.2a2

TABLE OF INDIVIDUAL MICROSCOPIC FINDINGS (AOFT)  
 DOSE GROUP : 04, 500.1 mg TOS/kg

ANIMAL NUMBER :

	61	62	63	64	65	66	67	68	69	70
	MK0	MK0	MK0	MK0	MK0	MK0	MK0	MK0	MK0	MK0
LIVER	+	-	+	+	-	+	+	-	-	-
- Vacuolation hepatoc.	.	.	1.	2.	.	1.	.	.	.	.
- Periarteritis, focal	.	.	.	.	.	.	1.	.	.	.
- Mononucl cells/EMH	1.	.	.	.	.	.	.	.	.	.
PANCREAS	-	-	-	+	-	-	-	-	-	-
- Mononucl cells focal	.	.	.	1.	.	.	.	.	.	.
KIDNEYS	-	-	+	+	+	+	+	-	+	+
- Tubular bas/dil foc.	.	.	( 1.	1. ( 1.	1. ( 1.	1. ( 1.	.	.	1. ( 1.	.
- Mononucl cells focal	.	.	.	.	( 1.	.	.	.	.	.
- Tubular dilatation	.	.	2.	1.	.	.	.	.	.	.
- Tubular hyaline cast	.	.	.	( 1.	.	.	.	.	.	.
URINARY BLADDER	-	-	-	-	-	-	+	-	-	-
- Mononucl cells focal	.	.	.	.	.	.	1.	.	.	.
TESTES	-	-	-	-	-	-	-	-	-	-
EPIDIDYMIDES	-	-	-	-	-	-	-	-	-	-
PROSTATE GLAND	-	-	-	-	-	-	-	-	-	-
SEMIN.VESICLE	-	-	-	-	-	-	-	-	-	-
PITUITARY GLAND	-	-	-	-	-	+	-	+	-	-
- Cyst(s), focal	.	.	.	.	.	P.	.	P.	.	.
THYROID GLAND	-	-	-	-	-	-	-	-	-	-
PARATHYROID GLANDS	-	-	-	-	-	-	-	-	-	0
ADRENAL GLANDS	-	-	-	-	-	-	-	+	-	-
- Vacuolation	.	.	.	.	.	.	.	2.	.	.
SPLEEN	-	-	-	-	-	-	-	-	-	-
THYMUS	-	+	+	+G	-	-	-	-	+	+
- Hemorrhage, focal	.	1.	1.	1.	.	.	.	.	1.	1.









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SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

ANIMAL HEADING DATA  
DOSE GROUP : 01, 0 mg TOS/kg  
-----

ANIMAL NUMBER	SEX M/F	DEFINED STATE	AND FINAL OF NECROPSY	TEST DAYS	FIRST DAY	AND LAST DAY UNDER TEST	DATE OF NECROPSY
1	M	K0	K0	91	22-MAY-07	20-AUG-07	20-AUG-07
2	M	K0	K0	91	22-MAY-07	20-AUG-07	20-AUG-07
3	M	K0	K0	91	22-MAY-07	20-AUG-07	20-AUG-07
4	M	K0	K0	91	22-MAY-07	20-AUG-07	20-AUG-07
5	M	K0	K0	91	22-MAY-07	20-AUG-07	20-AUG-07
6	M	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07
7	M	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07
8	M	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07
9	M	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07
10	M	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07
11	F	K0	K0	91	22-MAY-07	20-AUG-07	20-AUG-07
12	F	K0	K0	91	22-MAY-07	20-AUG-07	20-AUG-07
13	F	K0	K0	91	22-MAY-07	20-AUG-07	20-AUG-07
14	F	K0	K0	91	22-MAY-07	20-AUG-07	20-AUG-07
15	F	K0	K0	91	22-MAY-07	20-AUG-07	20-AUG-07
16	F	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07
17	F	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07
18	F	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07
19	F	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07
20	F	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07

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SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 01, 0 mg TOS/kg MALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 1  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

LUNG:  
-Alveolar macrophages, focal, grade 1  
LIVER:  
-Vacuolation, hepatocellular, multifocal, macrovesicular,  
grade 1  
THYMUS:  
-Hemorrhage, focal, grade 1  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 2  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

LUNG:  
-Mononuclear cells, focal, subpleural, grade 1  
KIDNEYS:  
-Tubular basophilia/dilatation, focal, unilateral, grade 1  
-Tubular hyaline casts, focal, unilateral, grade 1

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SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 01, 0 mg TOS/kg MALE  
-----

CONT./FF. ANIMAL NO. : 2  
.....

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 3  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

LUNG:

-Mononuclear cells, multifocal, subpleural/perivascular,  
grade 1

KIDNEYS:

-Tubular dilatation, diffuse, medulla, bilateral, grade 1

OPTIC NERVES:

Tissue not present for histologic examination

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.  
-----

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TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 01, 0 mg TOS/kg MALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 4  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

KIDNEYS:  
-Tubular basophilia/dilatation, focal, bilateral, grade 1  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 5  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

THYMUS:  
-Hemorrhage, focal, grade 1  
OPTIC NERVES:  
Tissue not present for histologic examination  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.  
-----

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-----  
TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 01, 0 mg TOS/kg MALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 6  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

HEART:

-Myofibre degeneration/regeneration, focal, grade 1  
associated with minimal accumulation of mononuclear cells.

KIDNEYS:

-Tubular basophilia/dilatation, focal, bilateral, grade 1

PITUITARY GLAND:

-Cyst(s), focal, pars distalis

OPTIC NERVES:

Tissue not present for histologic examination

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 7  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

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TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 01, 0 mg TOS/kg MALE  
-----

CONT./FF. ANIMAL NO. : 7  
.....

\* MICROSCOPIC FINDINGS

STOMACH GLANDULAR PART:  
-Mononuclear cells, focal, in submucosa, next to L.R., grade 1  
KIDNEYS:  
-Tubular basophilia/dilatation, focal, bilateral, grade 1  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 8  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

KIDNEYS:  
-Cyst, focal, medulla, unilateral  
TESTES:  
-Tubular atrophy/degeneration, multifocal, bilateral, grade 1  
THYMUS:  
-Hemorrhage, focal, grade 1  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.  
-----

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SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 01, 0 mg TOS/kg MALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 9  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

KIDNEYS:

-Tubular basophilia/dilatation, focal, bilateral, grade 1  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 10  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

LUNG:

-Mononuclear cells, multifocal, subpleural/perivascular,  
grade 1

LIVER:

-Periarteritis, focal, chronic, grade 1

KIDNEYS:

-Tubular basophilia/dilatation, focal, bilateral, grade 1

PITUITARY GLAND:

-Cyst(s), focal, pars intermedius

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SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 01, 0 mg TOS/kg MALE  
-----

CONT./FF. ANIMAL NO. : 10  
.....

OPTIC NERVES:

Tissue not present for histologic examination  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

-----

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SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 01, 0 mg TOS/kg FEMALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 11  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

LUNG:  
-Mononuclear cells, focal, subpleural, grade 1  
UTERUS:  
estrus  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 12  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

LUNG:  
-Mononuclear cells, focal, subpleural, grade 1  
UTERUS:  
diestrus  
PAROTID GLAND (RIGHT):  
Tissue not present for histologic examination  
OPTIC NERVES:  
Tissue not present for histologic examination

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SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 01, 0 mg TOS/kg FEMALE  
-----

CONT./FF. ANIMAL NO. : 12  
.....

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 13  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

KIDNEYS:  
-Tubular dilatation, diffuse, medulla, bilateral, grade 1  
UTERUS:  
diestrus  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 14  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

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SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 01, 0 mg TOS/kg FEMALE  
-----

CONT./FF. ANIMAL NO. : 14  
.....

\* MICROSCOPIC FINDINGS

KIDNEYS:

- Mononuclear cells, focal, interstitial, unilateral, grade 1
- Tubular dilatation, diffuse, medulla, bilateral, grade 1
- Mineralization, focal, at corticomedullary junction, unilateral, grade 1

UTERUS:

proestrus

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

-----  
\* STATE AT NECROPSY: K0

DAYS ON TEST : 91 \* ANIMAL NO. : 15  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

KIDNEYS:

- Tubular basophilia/dilatation, focal, unilateral, grade 1
- Tubular dilatation, diffuse, medulla, bilateral, grade 1

UTERUS:

estrus

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.  
-----

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SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 01, 0 mg TOS/kg FEMALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 16  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

LUNG:  
-Mononuclear cells, multifocal, perivascular, grade 1  
LIVER:  
-Mononuclear cells/extramedullary haematopoiesis, focal, grade 1  
UTERUS:  
metestrus  
MANDIBULAR LYMPH NODE, RIGHT:  
-Hemorrhage, focal, sinusoidal, grade 1  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 17  
.....

\* NECROPSY FINDINGS

THYMUS:  
01: Hemorrhage.  
NO OTHER NECROPSY OBSERVATIONS NOTED

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-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 01, 0 mg TOS/kg FEMALE  
-----

CONT./FF. ANIMAL NO. : 17  
.....

\* MICROSCOPIC FINDINGS

LUNG:  
-Mononuclear cells, focal, subpleural, grade 1  
KIDNEYS:  
-Tubular basophilia/dilatation, focal, bilateral, grade 1  
UTERUS:  
diestrus  
THYROID GLAND (BOTH LOBES):  
-Cyst, focal, unilateral  
THYMUS:  
-Hemorrhage, focal, grade 1  
This finding corresponds to necropsy observation no: 01.  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 18  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

LUNG:  
-Mononuclear cells, multifocal, subpleural/perivascular,  
grade 1  
UTERUS:  
estrus  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.  
-----

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SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 01, 0 mg TOS/kg FEMALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 19  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

UTERUS:  
estrus  
OPTIC NERVES:  
Tissue not present for histologic examination  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 20  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

LUNG:  
-Alveolar macrophages, focal, grade 1  
URINARY BLADDER:  
-Mononuclear cells, focal, in the submucosa, grade 1  
UTERUS:  
estrus  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.  
-----

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-----

ANIMAL HEADING DATA  
DOSE GROUP : 02, 50 mg TOS/kg  
-----

ANIMAL NUMBER	SEX M/F	DEFINED AND FINAL STATE OF NECROPSY	TEST DAYS	FIRST AND LAST DAY UNDER TEST	DATE OF NECROPSY
21	M	K0 +1	33	22-MAY-07 23-JUN-07	23-JUN-07
22	M	K0 K0	91	22-MAY-07 20-AUG-07	20-AUG-07
23	M	K0 K0	91	22-MAY-07 20-AUG-07	20-AUG-07
24	M	K0 K0	91	22-MAY-07 20-AUG-07	20-AUG-07
25	M	K0 K0	91	22-MAY-07 20-AUG-07	20-AUG-07
26	M	K0 K0	92	22-MAY-07 21-AUG-07	21-AUG-07
27	M	K0 K0	92	22-MAY-07 21-AUG-07	21-AUG-07
28	M	K0 K0	92	22-MAY-07 21-AUG-07	21-AUG-07
29	M	K0 K0	92	22-MAY-07 21-AUG-07	21-AUG-07
30	M	K0 K0	92	22-MAY-07 21-AUG-07	21-AUG-07
31	F	K0 K0	91	22-MAY-07 20-AUG-07	20-AUG-07
32	F	K0 K0	91	22-MAY-07 20-AUG-07	20-AUG-07
33	F	K0 K0	91	22-MAY-07 20-AUG-07	20-AUG-07
34	F	K0 K0	91	22-MAY-07 20-AUG-07	20-AUG-07
35	F	K0 K0	91	22-MAY-07 20-AUG-07	20-AUG-07
36	F	K0 K0	92	22-MAY-07 21-AUG-07	21-AUG-07
37	F	K0 K0	92	22-MAY-07 21-AUG-07	21-AUG-07
38	F	K0 K0	92	22-MAY-07 21-AUG-07	21-AUG-07
39	F	K0 K0	92	22-MAY-07 21-AUG-07	21-AUG-07
40	F	K0 K0	92	22-MAY-07 21-AUG-07	21-AUG-07

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-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 02, 50 mg TOS/kg MALE  
-----

\* STATE AT NECROPSY: K0/+1  
DAYS ON TEST : 33 \* ANIMAL NO. : 21  
.....

\* NECROPSY FINDINGS

GENERAL OBSERVATIONS:  
01: R dlig v ske i brysthulen  
NO OTHER NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

GENERAL OBSERVATIONS:  
Autolytic changes present in a proportion of tissues  
examined.  
No microscopic finding corresponding to necropsy observation no. 01.  
HEART:  
-Inflammatory cells, focal, mixed, in adnexal fat tissue,  
grade 1  
LUNG:  
-Alveolar hemorrhage, diffuse, grade 3  
LIVER:  
-Vacuolation, hepatocellular, multifocal, macrovescicular,  
grade 1  
THYMUS:  
-Hemorrhage, focal, grade 1  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

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-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 02, 50 mg TOS/kg MALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 22  
.....

\* NECROPSY FINDINGS  
NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS  
NO EXAMINATION REQUIRED.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 23  
.....

\* NECROPSY FINDINGS  
NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS  
NO EXAMINATION REQUIRED.  
-----

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-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 02, 50 mg TOS/kg MALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 24  
.....

\* NECROPSY FINDINGS  
NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS  
NO EXAMINATION REQUIRED.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 25  
.....

\* NECROPSY FINDINGS  
NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS  
NO EXAMINATION REQUIRED.  
-----

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SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 02, 50 mg TOS/kg MALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 26  
.....

\* NECROPSY FINDINGS  
NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS  
NO EXAMINATION REQUIRED.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 27  
.....

\* NECROPSY FINDINGS  
NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS  
NO EXAMINATION REQUIRED.  
-----

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SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 02, 50 mg TOS/kg MALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 28  
.....

\* NECROPSY FINDINGS  
NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS  
NO EXAMINATION REQUIRED.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 29  
.....

\* NECROPSY FINDINGS  
NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS  
NO EXAMINATION REQUIRED.  
-----

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SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 02, 50 mg TOS/kg MALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 30  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

-----

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SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 02, 50 mg TOS/kg FEMALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 31  
.....

\* NECROPSY FINDINGS  
NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS  
NO EXAMINATION REQUIRED.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 32  
.....

\* NECROPSY FINDINGS  
NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS  
NO EXAMINATION REQUIRED.  
-----

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SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 02, 50 mg TOS/kg FEMALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 33  
.....

\* NECROPSY FINDINGS  
NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS  
NO EXAMINATION REQUIRED.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 34  
.....

\* NECROPSY FINDINGS  
NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS  
NO EXAMINATION REQUIRED.  
-----

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-----  
TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 02, 50 mg TOS/kg FEMALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 35  
.....

\* NECROPSY FINDINGS  
NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS  
NO EXAMINATION REQUIRED.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 36  
.....

\* NECROPSY FINDINGS  
NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS  
NO EXAMINATION REQUIRED.  
-----

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-----  
TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 02, 50 mg TOS/kg FEMALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 37  
.....

\* NECROPSY FINDINGS  
NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS  
NO EXAMINATION REQUIRED.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 38  
.....

\* NECROPSY FINDINGS  
NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS  
NO EXAMINATION REQUIRED.  
-----

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-----  
TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 02, 50 mg TOS/kg FEMALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 39  
.....

\* NECROPSY FINDINGS  
NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS  
NO EXAMINATION REQUIRED.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 40  
.....

\* NECROPSY FINDINGS  
NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS  
NO EXAMINATION REQUIRED.  
-----

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-----  
TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

ANIMAL HEADING DATA  
DOSE GROUP : 03, 165.1 mg TOS/kg  
-----

ANIMAL NUMBER	SEX M/F	DEFINED AND STATE	FINAL OF NECROPSY	TEST DAYS	FIRST AND DAY	LAST UNDER TEST	DATE OF NECROPSY
41	M	K0	K0	91	22-MAY-07	20-AUG-07	20-AUG-07
42	M	K0	+2	25	22-MAY-07	15-JUN-07	15-JUN-07
43	M	K0	K0	91	22-MAY-07	20-AUG-07	20-AUG-07
44	M	K0	K0	91	22-MAY-07	20-AUG-07	20-AUG-07
45	M	K0	K0	91	22-MAY-07	20-AUG-07	20-AUG-07
46	M	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07
47	M	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07
48	M	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07
49	M	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07
50	M	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07
51	F	K0	+1	7	22-MAY-07	28-MAY-07	01-JUN-07
52	F	K0	K0	91	22-MAY-07	20-AUG-07	20-AUG-07
53	F	K0	K0	91	22-MAY-07	20-AUG-07	20-AUG-07
54	F	K0	K0	91	22-MAY-07	20-AUG-07	20-AUG-07
55	F	K0	+1	8	22-MAY-07	29-MAY-07	01-JUN-07
56	F	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07
57	F	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07
58	F	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07
59	F	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07
60	F	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07

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-----  
TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 03, 165.1 mg TOS/kg MALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 41  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

-----  
\* STATE AT NECROPSY: K0/+2  
DAYS ON TEST : 25 \* ANIMAL NO. : 42  
.....

\* NECROPSY FINDINGS

GENERAL OBSERVATIONS:

01: Animal autolytic.

BODY CAVITIES:

01: Peritoneum, Abdominal cavity: Edema.

02: Chest cavity, Pleura: Edema, Contains reddish-watery fluid.

NO OTHER NECROPSY OBSERVATIONS NOTED

\* MICROSCOPIC FINDINGS

GENERAL OBSERVATIONS:

Autolytic changes present in a proportion of tissues examined.

No microscopic finding corresponding to necropsy observation no. 01.

LUNG:

-Alveolar hemorrhage, diffuse, grade 3

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-----  
TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 03, 165.1 mg TOS/kg MALE  
-----

CONT./FF. ANIMAL NO. : 42  
.....

BODY CAVITIES:

No microscopic finding corresponding to necropsy observation  
no. 01,02.

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 43  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 44  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

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-----  
TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 03, 165.1 mg TOS/kg MALE  
-----

CONT./FF. ANIMAL NO. : 44  
.....

\* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 45  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 46  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

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-----  
TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 03, 165.1 mg TOS/kg MALE  
-----

CONT./FF. ANIMAL NO. : 46  
.....

\* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

-----  
\* STATE AT NECROPSY: K0

DAYS ON TEST : 92 \* ANIMAL NO. : 47  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

-----  
\* STATE AT NECROPSY: K0

DAYS ON TEST : 92 \* ANIMAL NO. : 48  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

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-----  
TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 03, 165.1 mg TOS/kg MALE  
-----

CONT./FF. ANIMAL NO. : 48  
.....

\* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 49  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 50  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

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TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 03, 165.1 mg TOS/kg MALE  
-----

CONT./FF. ANIMAL NO. : 50  
.....

\* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.  
-----

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TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 03, 165.1 mg TOS/kg FEMALE  
-----

\* STATE AT NECROPSY: K0/+1  
DAYS ON TEST : 7 \* ANIMAL NO. : 51  
.....

\* NECROPSY FINDINGS

LUNG:  
01: All lobes: discoloration, red.  
THYMUS:  
01: Discoloration: Red.  
BODY CAVITIES:  
01: Chest cavity: Containing blood/blood clots.  
NO OTHER NECROPSY OBSERVATIONS NOTED

\* MICROSCOPIC FINDINGS

GENERAL OBSERVATIONS:  
Autolytic changes present in a proportion of tissues examined.  
LUNG:  
-Alveolar hemorrhage, diffuse, grade 3  
This finding corresponds to necropsy observation no: 01.  
UTERUS:  
proestrus  
PARATHYROID GLANDS:  
Tissue not present for histologic examination  
THYMUS:  
-Hemorrhage, focal, grade 1  
This finding corresponds to necropsy observation no: 01.  
BODY CAVITIES:  
No microscopic finding corresponding to necropsy observation no. 01.  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

-----

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TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 03, 165.1 mg TOS/kg FEMALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 52  
.....

\* NECROPSY FINDINGS  
NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS  
NO EXAMINATION REQUIRED.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 53  
.....

\* NECROPSY FINDINGS  
NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS  
NO EXAMINATION REQUIRED.  
-----

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TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 03, 165.1 mg TOS/kg FEMALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 54  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.

-----  
\* STATE AT NECROPSY: K0/+1  
DAYS ON TEST : 8 \* ANIMAL NO. : 55  
.....

\* NECROPSY FINDINGS

LUNG:  
01: All lobes: discoloration, red.  
ESOPHAGUS:  
01: Perforation.  
BODY CAVITIES:  
01: Chest cavity: Containing blood/blood clots.  
NO OTHER NECROPSY OBSERVATIONS NOTED

\* MICROSCOPIC FINDINGS

LUNG:  
-Alveolar hemorrhage, diffuse, grade 3  
This finding corresponds to necropsy observation no: 01.  
ESOPHAGUS:  
No microscopic finding corresponding to necropsy observation no. 01.

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TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 03, 165.1 mg TOS/kg FEMALE  
-----

CONT./FF. ANIMAL NO. : 55  
.....

LIVER:

-Vacuolation, hepatocellular, multifocal, macrovesicular,  
grade 2

KIDNEYS:

-Tubular basophilia/dilatation, focal, unilateral, grade 1

UTERUS:

proestrus going towards estrus

THYMUS:

-Hemorrhage, focal, grade 1

BODY CAVITIES:

No microscopic finding corresponding to necropsy observation no. 01.  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

-----  
\* STATE AT NECROPSY: K0

DAYS ON TEST : 92

\* ANIMAL NO. : 56  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

NO EXAMINATION REQUIRED.  
-----

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TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 03, 165.1 mg TOS/kg FEMALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 57  
.....

\* NECROPSY FINDINGS  
NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS  
NO EXAMINATION REQUIRED.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 58  
.....

\* NECROPSY FINDINGS  
NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS  
NO EXAMINATION REQUIRED.  
-----

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TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 03, 165.1 mg TOS/kg FEMALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 59  
.....

\* NECROPSY FINDINGS  
NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS  
NO EXAMINATION REQUIRED.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 60  
.....

\* NECROPSY FINDINGS  
NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS  
NO EXAMINATION REQUIRED.  
-----

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TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

ANIMAL HEADING DATA  
DOSE GROUP : 04, 500.1 mg TOS/kg  
-----

ANIMAL NUMBER	SEX M/F	DEFINED AND STATE	FINAL OF NECROPSY	TEST DAYS	FIRST DAY	AND LAST UNDER TEST	DATE OF NECROPSY
61	M	K0	K0	91	22-MAY-07	20-AUG-07	20-AUG-07
62	M	K0	K0	91	22-MAY-07	20-AUG-07	20-AUG-07
63	M	K0	K0	91	22-MAY-07	20-AUG-07	20-AUG-07
64	M	K0	K0	91	22-MAY-07	20-AUG-07	20-AUG-07
65	M	K0	K0	91	22-MAY-07	20-AUG-07	20-AUG-07
66	M	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07
67	M	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07
68	M	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07
69	M	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07
70	M	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07
71	F	K0	K0	91	22-MAY-07	20-AUG-07	20-AUG-07
72	F	K0	K0	91	22-MAY-07	20-AUG-07	20-AUG-07
73	F	K0	K0	91	22-MAY-07	20-AUG-07	20-AUG-07
74	F	K0	K0	91	22-MAY-07	20-AUG-07	20-AUG-07
75	F	K0	K0	91	22-MAY-07	20-AUG-07	20-AUG-07
76	F	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07
77	F	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07
78	F	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07
79	F	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07
80	F	K0	K0	92	22-MAY-07	21-AUG-07	21-AUG-07

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SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 04, 500.1 mg TOS/kg MALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 61  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

LIVER:  
-Mononuclear cells/extramedullary haematopoiesis, focal, grade 1  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 62  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

THYMUS:  
-Hemorrhage, focal, grade 1  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.  
-----

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TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 04, 500.1 mg TOS/kg MALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 63  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

LIVER:

-Vacuolation, hepatocellular, multifocal, macrovescicular,  
grade 1

KIDNEYS:

-Tubular basophilia/dilatation, focal, unilateral, grade 1  
-Tubular dilatation, diffuse, medulla/papilla, bilateral,  
grade 2

THYMUS:

-Hemorrhage, focal, grade 1

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 64  
.....

\* NECROPSY FINDINGS

THYMUS:

01: Hemorrhage.

NO OTHER NECROPSY OBSERVATIONS NOTED

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-----  
TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 04, 500.1 mg TOS/kg MALE  
-----

CONT./FF. ANIMAL NO. : 64  
.....

\* MICROSCOPIC FINDINGS

LUNG:  
-Mononuclear cells, focal, subpleural, grade 1  
LIVER:  
-Vacuolation, hepatocellular, multifocal, macrovesicular,  
grade 2  
PANCREAS:  
-Mononuclear cells, multifocal, interstitial, grade 1  
KIDNEYS:  
-Tubular basophilia/dilatation, focal, bilateral, grade 1  
-Tubular dilatation, diffuse, medulla, bilateral, grade 1  
-Tubular hyaline casts, focal, unilateral, grade 1  
THYMUS:  
-Hemorrhage, focal, grade 1  
This finding corresponds to necropsy observation no: 01.  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 65  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

LUNG:  
-Osseous metaplasia, focal  
KIDNEYS:  
-Tubular basophilia/dilatation, focal, unilateral, grade 1  
-Mononuclear cells, focal, interstitial, unilateral, grade 1

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-----  
TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 04, 500.1 mg TOS/kg MALE  
-----

CONT./FF. ANIMAL NO. : 65  
.....

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 66  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

LUNG:  
-Alveolar hemorrhage, focal, grade 1  
LIVER:  
-Vacuolation, hepatocellular, multifocal, macrovescicular,  
grade 1  
KIDNEYS:  
-Tubular basophilia/dilatation, focal, bilateral, grade 1  
PITUITARY GLAND:  
-Cyst(s), focal, pars distalis  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.  
-----

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-----  
TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 04, 500.1 mg TOS/kg MALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 67  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

LIVER:  
-Periarteritis, focal, chronic, grade 1  
KIDNEYS:  
-Tubular basophilia/dilatation, focal, unilateral, grade 1  
URINARY BLADDER:  
-Mononuclear cells, focal, in the submucosa, grade 1  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 68  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

LUNG:  
-Mononuclear cells, focal, subpleural, grade 1  
PITUITARY GLAND:  
-Cyst(s), focal, pars distalis  
ADRENAL GLANDS:  
-Vacuolation, diffuse, fasciculata & reticularis, macroves.,  
bilateral, grade 2

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-----  
TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 04, 500.1 mg TOS/kg MALE  
-----

CONT./FF. ANIMAL NO. : 68  
.....

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 69  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

LUNG:

-Mononuclear cells, multifocal, subpleural/perivascular,  
grade 1

KIDNEYS:

-Tubular basophilia/dilatation, focal, bilateral, grade 1

THYMUS:

-Hemorrhage, focal, grade 1

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.  
-----

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PATHOLOGY REPORT PAGE : 73/ 80  
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-----  
TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 04, 500.1 mg TOS/kg MALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 70  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

KIDNEYS:

-Tubular basophilia/dilatation, focal, unilateral, grade 1

PARATHYROID GLANDS:

Tissue not present for histologic examination

THYMUS:

-Hemorrhage, focal, grade 1

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

-----

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Sponsor Ref No 20076029

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PATHOLOGY REPORT PAGE : 74/ 80  
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-----  
TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 04, 500.1 mg TOS/kg FEMALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 71  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

KIDNEYS:  
-Tubular dilatation, diffuse, medulla, unilateral, grade 1  
UTERUS:  
metestrus  
OPTIC NERVES:  
Tissue not present for histologic examination  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 72  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

KIDNEYS:  
-Tubular dilatation, diffuse, medulla, bilateral, grade 1  
UTERUS:  
metestrus  
THYMUS:  
-Hemorrhage, focal, grade 1

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-----  
TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 04, 500.1 mg TOS/kg FEMALE  
-----

CONT./FF. ANIMAL NO. : 72  
.....

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 73  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

KIDNEYS:

- Tubular dilatation, diffuse, medulla, unilateral, grade 2
- Tubular hyaline casts, focal, unilateral, grade 1
- Pelvic dilatation, unilateral, grade 1

UTERUS:

metestrus

OPTIC NERVES:

Tissue not present for histologic examination

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.  
-----

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-----  
TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 04, 500.1 mg TOS/kg FEMALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 74  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

KIDNEYS:  
-Mononuclear cells, focal, interstitial, unilateral, grade 2  
URINARY BLADDER:  
-Mononuclear cells, focal, in the submucosa, grade 1  
UTERUS:  
metestrus  
PITUITARY GLAND:  
-Cyst(s), focal, pars distalis  
OPTIC NERVES:  
Tissue not present for histologic examination  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 91 \* ANIMAL NO. : 75  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

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-----  
TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 04, 500.1 mg TOS/kg FEMALE  
-----

CONT./FF. ANIMAL NO. : 75  
.....

\* MICROSCOPIC FINDINGS

KIDNEYS:

- Mononuclear cells, focal, interstitial, unilateral, grade 1
- Tubular dilatation, diffuse, medulla, unilateral, grade 2

UTERUS:

estrus

THYMUS:

- Hemorrhage, focal, grade 1

SUBLINGUAL GLAND (RIGHT):

- Inflammation, focal, subacute, ductular, grade 2

OPTIC NERVES:

Tissue not present for histologic examination

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

-----  
\* STATE AT NECROPSY: K0

DAYS ON TEST : 92

\* ANIMAL NO. : 76  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

LUNG:

- Mononuclear cells, focal, subpleural, grade 1

PANCREAS:

- Atrophy exocrine part, focal, grade 1

UTERUS:

metestrus

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PATHOLOGY REPORT PAGE : 78/ 80  
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-----  
TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 04, 500.1 mg TOS/kg FEMALE  
-----

CONT./FF. ANIMAL NO. : 76  
.....

ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 77  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

LUNG:  
-Mononuclear cells, focal, subpleural, grade 1  
UTERUS:  
diestrus  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 78  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

Study No: 66063  
Sponsor Ref No 20076029

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PATHOLOGY REPORT PAGE : 79/ 80  
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-----  
TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 04, 500.1 mg TOS/kg FEMALE  
-----

CONT./FF. ANIMAL NO. : 78  
.....

\* MICROSCOPIC FINDINGS

UTERUS:  
proestrus  
OPTIC NERVES:  
Tissue not present for histologic examination  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

-----  
\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 79  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

LUNG:  
-Mononuclear cells, focal, perivascular, grade 1  
UTERUS:  
proestrus going towards estrus  
PAROTID GLAND (RIGHT):  
-Mononuclear cells, focal, grade 1  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.  
-----

Study No: 66063  
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-----  
TEST ARTICLE : Serine endopeptidase (PPA 26797) PATHOL. NO.: 66063 GN  
TEST SYSTEM : RAT, 13-WEEK, ORAL DATE : 03-JUL-09  
SPONSOR : Novozymes A/S PathData@System V6.2a2  
-----

TEXT OF GROSS AND MICROSCOPIC FINDINGS  
DOSE GROUP : 04, 500.1 mg TOS/kg FEMALE  
-----

\* STATE AT NECROPSY: K0  
DAYS ON TEST : 92 \* ANIMAL NO. : 80  
.....

\* NECROPSY FINDINGS

NO NECROPSY OBSERVATIONS NOTED.

\* MICROSCOPIC FINDINGS

LIVER:  
-Mononuclear cells/extramedullary haematopoiesis, focal, grade 1  
KIDNEYS:  
-Tubular basophilia/dilatation, focal, unilateral, grade 1  
UTERUS:  
diestrus  
ALL OTHER PROTOCOL TISSUES WITHOUT PATHOLOGIC FINDINGS.

-----

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**Appendix II      Analysis of dose formulation  
(9 pages, excl. this cover page)**

**Process Support Laboratories  
Enzyme Analytical Laboratory**

MGhi  
2009-06-25  
Luna no. 2007-38542-02

**LAB Scantox study no: 66063  
Novozymes reference no.: 20076029**

**Investigation Report Amended  
Serine Endopeptidase, PPA 26797  
A 13-Week oral (Gavage) Toxicity Study in Rats**

**Analysis of dose formulations on samples returned from LAB Scantox  
(content check)**

**Content:**

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<b>2. Quality Assurance statement.....</b>	<b>3</b>
<b>3. General Information.....</b>	<b>4</b>
<b>4. Purpose.....</b>	<b>5</b>
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<b>8. Results and discussion.....</b>	<b>7</b>
<b>9. Conclusion.....</b>	<b>9</b>
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## 1. GLP Compliance

Ref. no. 20076029:

This investigation was conducted at the Process Support Laboratories, Enzyme Analytical Laboratory, Novozymes A/S, in compliance with OECD principles of Good Laboratory Practice, ENV/MC/CHEM (98) 17.

This investigation was first reported on 20070918. This amended final investigation report was issued on 20090706 for the following reasons:

For confidentiality reasons, the internal Novozymes name, used in the development phase and the toxicological studies of the enzyme, has been removed from the present investigation report. Instead, the enzyme is referred to as *Serine Endopeptidase* throughout the report.

For confidentiality reasons the title of PSL-SM-0609.01-D version 6.0, which is referred to in chapter 6, has been removed as it contains the internal Novozymes name.

There has been a change in the Principal Investigator for the amended investigation report since the original Principal Investigator is not working for Novozymes anymore. The new Principal Investigator was the manager of the former Principal Investigator.

Two minor editorial corrections were necessary

- 1) a reference to chapter 7 was added in the contents table on the front page
- 2) "Non-exciting" was changed to "Non-existing" in the caption of Table 1.
- 3) The Study number in the GLP compliance statement was corrected to the reference number.

The changes in the amended Investigation Report affect neither the original data nor the original conclusions.

2009 0706

Date

  
Principal Investigator

## QUALITY ASSURANCE STATEMENT

2

Report: Serine Endopeptidase, PPA 26797  
A 13-Week oral (Gavage) Toxicity Study in Rats  
Analysis of dose formulations on samples from LaB Scantox

STUDY NUMBER 66063

REFERENCE NUMBER 20076029

The conduct of this study has been subject to appropriate inspections and the report has been reviewed according to the relevant Standard Operation Procedures of Novozymes A/S Quality Assurance.

Inspection/Audit	Dates of inspection	Dates of Audit Report signed by Principle Investigator	Dates of Audit Report signed by Management
Transfer from Tox.	31 AUG 2007	4 SEP 2007	4 SEP 2007
Report	14 SEP 2007	18 SEP 2007	18 SEP 2007
Report - Amended	2 JUL 2009	6 JUL 2009	6 JUL 2009

6 Jul 2009  
Date



Quality Assurance

### 3 General information

**Principal Investigator:**

[REDACTED]  
Novozymes A/S  
Enzyme Analytical Laboratory (EAL)  
Krogshøjvej 36, 6E1.22  
DK-2880 Bagsværd

[REDACTED]

**Sponsor Monitor:**

[REDACTED]  
Safety & Toxicology  
Novozymes A/S  
Krogshøjvej 36, 2880 Bagsværd, Denmark

[REDACTED]

**Study Director:**

[REDACTED]

**Laboratory:**

Enzyme Analytical Laboratory (EAL)  
Process Support Laboratories  
Novozymes A/S  
Krogshøjvej 36, 2880 Bagsværd

**Personel:**

Laboratory Technician: Birgitte Skaarup (BgSk)

Approved by: 20090706

Date

[REDACTED]

Principal Investigator

#### 4 Purpose

The purpose of this investigation is to determine whether the Serine Endopeptidase activity (PROT/g) in the dose solutions from week 1, 6 and 13 are approximately equal and to check if the activity of the 100% dose solutions complies relatively with the enzyme activity of the tox-batch.

Content check analysis is required as part of the OECD guidelines for oral toxicity studies.

#### 5 Sample Handling

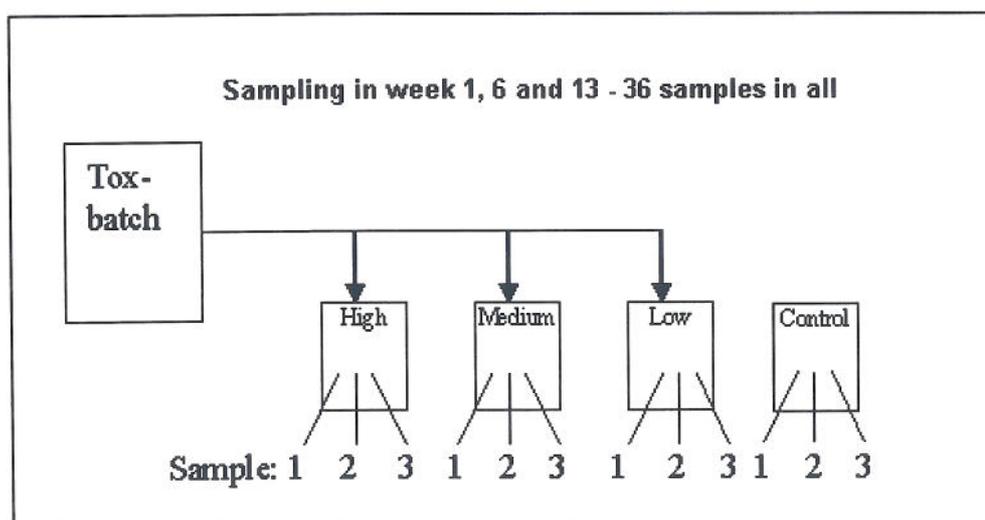
##### Sample description

During the study a total of 36 samples were taken out for analysis of activity:

There were four groups:

- High activity (approx. 100%)
- Medium activity (approx. 33%)
- Low activity (approx. 10%)
- Control group (approx. 0%)

During week 1, 6 and 13, three samples of 10 ml were taken from each group and labelled "1", "2" and "3", as illustrated below.



First all samples labelled "1" were analysed. More details about the schedule for the analytical phase are found in the current version of PSL-SP-0107.01-D "Aktivitetsbestemmelser på teststofprøver fra eksterne studier (GLP)".

Expected enzyme activities:

High activity (approx. 100%) contains:	54600 PROT/g
Medium activity (approx. 33%) contains:	18200 PROT/g
Low activity (approx. 10%) contains:	5460 PROT/g
Control group (approx. 0%) contains:	0 PROT/g

### **Sample transportation and registration**

Samples from LAB Scantox were sent directly to Safety & Toxicology in Novozymes Att. Ditte Sidelmann Brinch, where the samples were registered. The samples were stored frozen (-18°C) by Safety & Toxicology until transfer to EAL for analysis.

All 36 samples were received 20070831 at EAL

### **Storage of samples for analysis**

After registration in EAL the samples were stored frozen (-18°C) until analysis.

### **Sample defrost**

The "1" samples were defrosted at room temperature 20070904.

### **Date of analysis**

Analysis of the "1" samples were carried out 20070904.

## 6 Method

The analysis was performed according to PSL-SM-0609.01-D version 6.0 (=EB-SM-0609.02)

Serine Endopeptidase hydrolyses the substrate Suc-Ala-Ala-Pro-Phe-pNA. The release of yellow pNA (p-NitroAniline) leads to a rise in absorbance at 405 nm which is proportional to the enzyme activity

The samples were analysed as 2 weightings on 1 standard curve as specified for GLP samples in:

PSL-SP-0598.01 -D - version 4.0

PSL-MSK-0598SP02 - version 10.0

## 7 Deviations

No deviations to report

## 8 Results and discussion

Only "1" samples from the high, medium, low and control group were analysed.

The results were analysed according to PSL-SP-0107.01-D - version 6.0.

All calculations were carried out using the work sheet PSL-AS-0022 Version 3.0

**Table 1.** Analysis results of each sample for the dose groups High, Medium and Low, given in PROT/g. Non-existing results are marked with "-".

Week	Sample No.	High	Medium	Low
1	1	52200	18400	5400
	2	-	-	-
	3	-	-	-
6	1	55300	17600	5240
	2	-	-	-
	3	-	-	-
13	1	52500	17500	5670
	2	-	-	-
	3	-	-	-

Expected activities in PROT/g: High (54600); Medium (18200), Low (5460).

No activity above the detection limit was found for the Control group.

### Investigation of whether the activity is constant during the study for groups High, Medium and Low

**Table 2.** Approximate 95% confidence intervals for ratios between activity in week 6 and 13. Reference: week 1.

Group	Week	Lower Limit	Upper Limit	Is there significant difference?
High	6	0,96	1,17	No
	13	0,91	1,11	No
Medium	6	0,86	1,05	No
	13	0,86	1,04	No
Low	6	0,88	1,07	No
	13	0,95	1,16	No

No significant difference was found between the two weeks.

### Investigation of whether the activity is approximately the same for group High and the Tox-batch.

**Table 3.** Mean activity per group and week for groups High, Medium and Low.

Week	Group High	Group Medium	Group Low
1	52200	18400	5400
6	55300	17600	5240
13	52500	17500	5670

**Table 4.** Mean activity per group for groups High, Medium and Low.

Group High	Group Medium	Group Low
55300	17800	5430

**Table 5.** 95% confidence interval for ratio between Mean of group High and Tox-batch (Group High/Tox-batch)

Analysis result for Tox-batch	Number of standard curves for Tox-batch ( $K_{Tox}$ )	Number of weighings per standard curve for Tox-batch ( $N_{Tox}$ )	Mean of group High	Lower Limit	Upper Limit	Is there significant difference?
54600	3	2	53300	0,88	1,09	No

No significant differences were found between group High and the Tox-batch.

The mean activity for each group is listed in Table 4.

## **9 Conclusion**

The Serine Endopeptidase activities (PROT/g) in the dose solutions from week 1, 6 and 13 were approximately equal and the activity of the 100% dose solutions complies relatively with the enzyme activity of the tox-batch.

No significant difference was found between week 1, 6 and 13.

No significant differences were found between group High and the Tox-batch

## **10 Quality assurance**

Contribution, experiments, data and Investigation Report have been subject to audit by Novozymes QA (GLP)

## **11 Archive**

Original contribution, Investigation Plan, raw data or exact copies and the Investigation report are archived in Novozymes QM Central Archive by Novozymes Safety & Toxicology. A copy of this Investigation Report is distributed to LAB Scantox A/S for inclusion in the final report as an addendum.

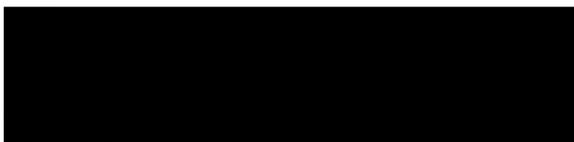
## APPENDIX 6

Non-CCI version

Does not include confidential commercial information

**Serine protease from *Nocardiosis prasina*  
produced by a genetically modified strain of *Bacillus  
licheniformis***

**Novozymes A/S**  
July 16<sup>th</sup> 2014



Regulatory Affairs



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## Appendix 6

### Non-CCI version

***Elements in Appendix 6 that are to be treated as confidential commercial information (CCI) are marked with a red box in the CCI version. The confidential information has been removed from the non-CCI version. Grey colour has been used for the applied redactions.***

### Documentation regarding the production strain

1. Detailed description of the construction of the genetically modified production strain
2. Description of general methods.
3. Annotated DNA sequence *amyL* region
4. Annotated DNA sequence *xyIA* region
5. Genetic stability of the production strain (Southern blot)

## References for Appendix 6

Gryczan TJ, Contente S, Dubnau D (1978) Characterization of *Staphylococcus aureus* Plasmids Introduced by Transformation into *Bacillus subtilis*. *Journal of Bacteriology*, 134 (1), 318-329.

Horinouchi S, Weisblum B (1982) Nucleotide Sequence and Functional Map of pE194, a Plasmid that Specifies Inducible Resistance to Macrolide, Lincosamide, and Streptogramin Type B Antibiotics. *Journal of Bacteriology*, 150 (2), 804-814.

## Appendix 6.1

### Detailed description of the construction of the genetically modified production strain

#### 6.1.1. The host organism

##### Taxonomy

The parental strain Ca63 is a natural isolate and the taxonomic classification is as followed:

Name:	<i>Bacillus licheniformis</i>
Phylum:	Firmicutes
Class:	Bacilli
Order:	<i>Bacillales</i>
Genus:	<i>Bacillus</i>
Species:	<i>licheniformis</i>

The classification was confirmed by Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH and deposited as DSM 9552 (equals to ATCC 9789).

##### Genetic modifications

Host strain *B. licheniformis* SJ6370 was constructed from parental strain Ca63 [REDACTED] (Fig. 1). The modifications are briefly described below. The general methods used for engineering the strain are described in Appendix 6.2. The loci modified during the GM steps leading from parental strain Ca63 to host strain SJ6370 are listed in Table 1.

Locus	Function
<i>aprL</i>	Alkaline protease
<i>amyL</i>	Alpha-amylase
<i>mprL</i>	Glu-specific protease
<i>xyIA</i>	Xylose isomerase

**Table 1:** Loci modified in GM steps during construction of host strain SJ6370





No antibiotic marker genes were introduced into the host strain as a result of these genetic modifications.

### 6.1.2 Origin and donor of vector and inserts

#### The enzyme gene

The serine protease is encoded by a tandem gene construct inserted at the *amyL* and *xylA* loci in the chromosome. Each tandem gene construct consists of two genes [REDACTED] namely the serine protease from *Nocardiopsis prasina* [REDACTED]. In total, four copies of the gene encoding the [REDACTED] are present in the production strain and these are hereafter collectively referred to as [REDACTED].

#### Promoter

During construction of the host strain SJ6370, a triple tandem promoter construct is inserted at the *amyL* and *xylA* locus, respectively. This triple tandem promoter construct consists of three promoter fragments, originating from three different donor organisms.

The three promoters are:



#### Terminator

The transcriptional terminator (*amyL* term) inserted downstream of the serine protease construct at both the *amyL* and *xylA* locus is from the *Bacillus licheniformis* DSM 9552.

#### Vector/insert

Vectors used are composed of elements from plasmids pUB110 (Gryczan *et al*, 1978) and pE194 (Horinouchi, S. and Weisblum, B., 1982).

### 6.1.2. Introduced genetic sequence

#### Construction of production strain from host strain SJ6370

The production strain *B. licheniformis* [REDACTED] was constructed from the host strain *B. licheniformis* SJ6370 using the double homologous recombination strategy explained in the general methods (Appendix 6.2). Two different plasmids containing the tandem gene construct described in the section above were used to insert a total of four copies of the [REDACTED] downstream of the modified triple tandem promoter [REDACTED] present at the *amyL* and *xylA* loci. Further, the ribosome binding site (RBS) in front of

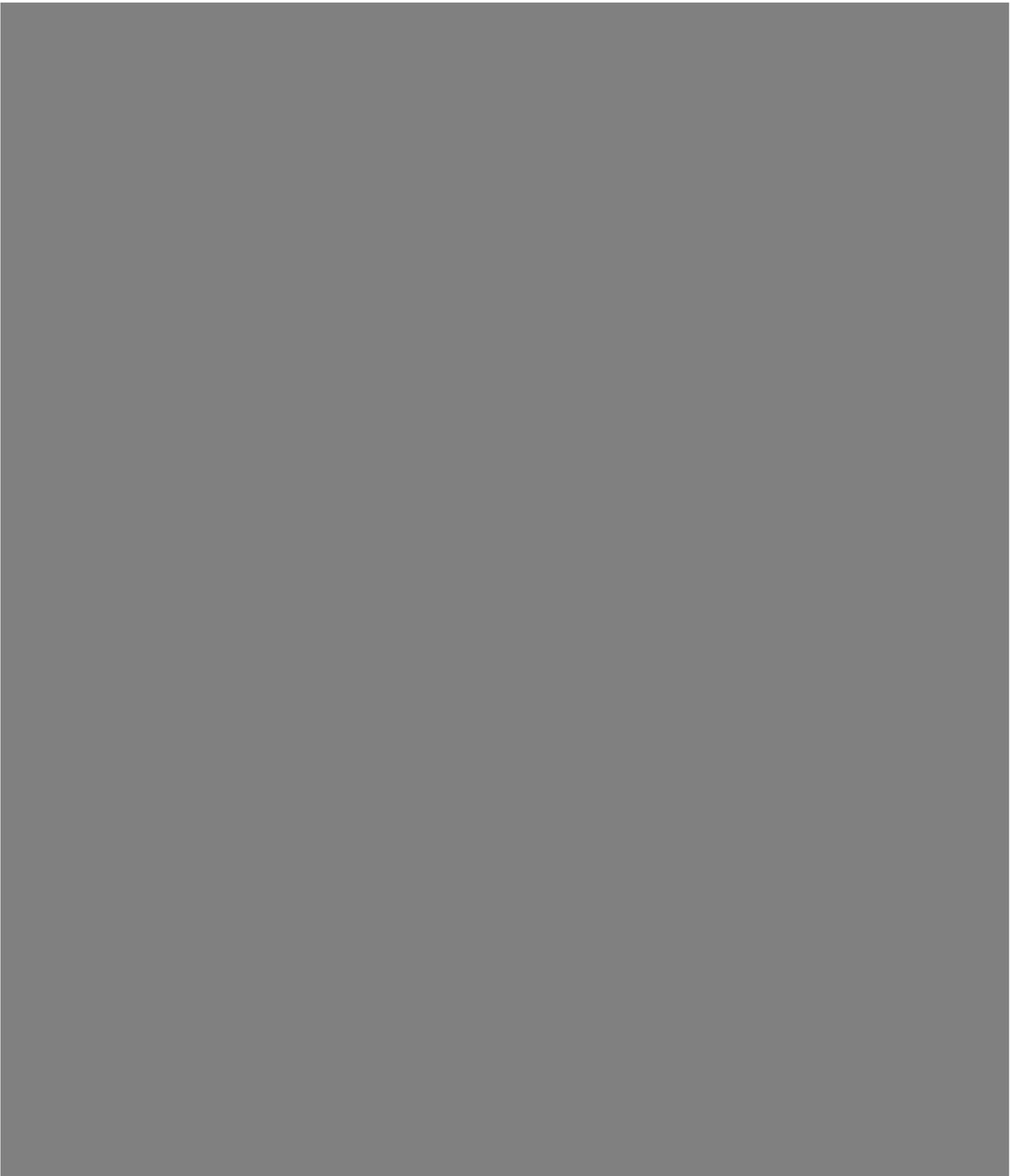
■ was replaced with a non-functional RBS version leading to an abolished production of this background protein.

Fig. 2 presents the pedigree leading from host strain SJ6370 to the final production strain, including the genetic change introduced in each step and the plasmids used in the process.

The methods used during strain construction are described in Appendix 6.2.







To confirm the number and position of the four [REDACTED] copies that have been inserted in the production strain [REDACTED], Southern blot analysis was performed on strains in the pedigree leading from host strain to the production strain with a probe specific for [REDACTED] genes. Bands of the expected sizes were obtained, confirming that all four [REDACTED] copies are inserted at the target loci in a correct manner.

To verify that the intended modifications have been introduced in *amyL* and *xyIA*, the DNA sequences of these loci were determined in the final production strain. The annotated sequence of the *amyL* locus containing the serine protease genes is given in Appendix 6.3 whereas the annotated sequence of the *xyIA* locus containing the serine protease genes is given in Appendix 6.4. The sequences were determined as described in the general methods (Appendix 6.2) and were found to match the expected sequences.

To demonstrate that no ARM genes from these used plasmids were present in the final production strain, Southern blot analysis was performed on chromosomal DNA from this strain using different probes designed to recognize relevant target sequences (ARMs) of these vectors. The results showed absence of ARM genes in the final production strain.

### **6.1.3. Description of the production organism**

The chromosome of the final production strain has been modified by recombinant DNA techniques at five different positions relative to the non-recombinant strain Si3. These positions are

- A) The position of the gene encoding an alkaline protease where a deletion was introduced.
- B) The position of the gene encoding a glu-specific protease, where a deletion was introduced.
- C) The position of the gene encoding a background protein, [REDACTED], where the ribosome binding site was modified.
- D) The position of the gene encoding the alpha-amylase, *amyL*, where the expression cassette with the tandem serine protease gene construct was inserted.
- E) The position of the gene encoding the xylose isomerase, *xyIA*, where the expression cassette with the tandem serine protease gene construct was inserted.

The resulting strain was subsequently subjected to classical mutagenesis and a production strain [REDACTED] giving a high yield was selected.

#### Identity and taxonomy of production organism

The production strain for the serine protease is a *Bacillus licheniformis* carrying four genes encoding the serine protease from *Nocardioopsis prasina*. The strain is derived from a sporulation deficient *Bacillus licheniformis*, as described above.

#### Genetic stability and mobilization and conjugation capability

The inserted recombinant DNA is genetically stable during fermentation, as the inserted DNA is integrated into the chromosome.

The genetic stability of the production strain was tested at large-scale fermentation. The strain stability during fermentation was analyzed by Southern blotting. No instability of the strain was observed (Appendix 6.5).

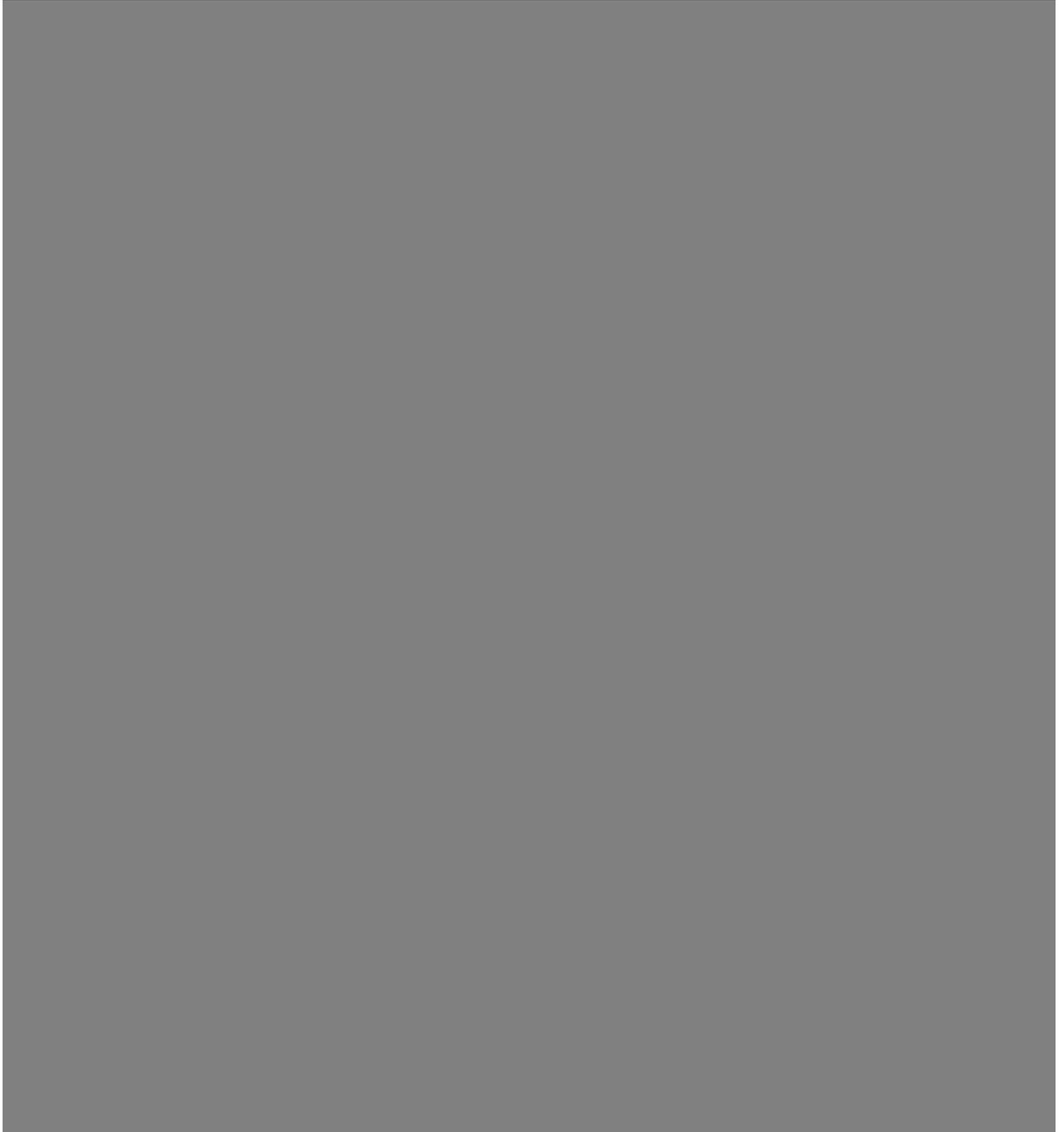
As the insert is chromosomally integrated and lacks a functional origin of replication, it cannot be transferred by conjugation to other organisms, nor can fragments replicate autonomously.

#### Antibiotic resistance gene

No functional antibiotic resistance genes were left in the strain as a result of the genetic modifications.

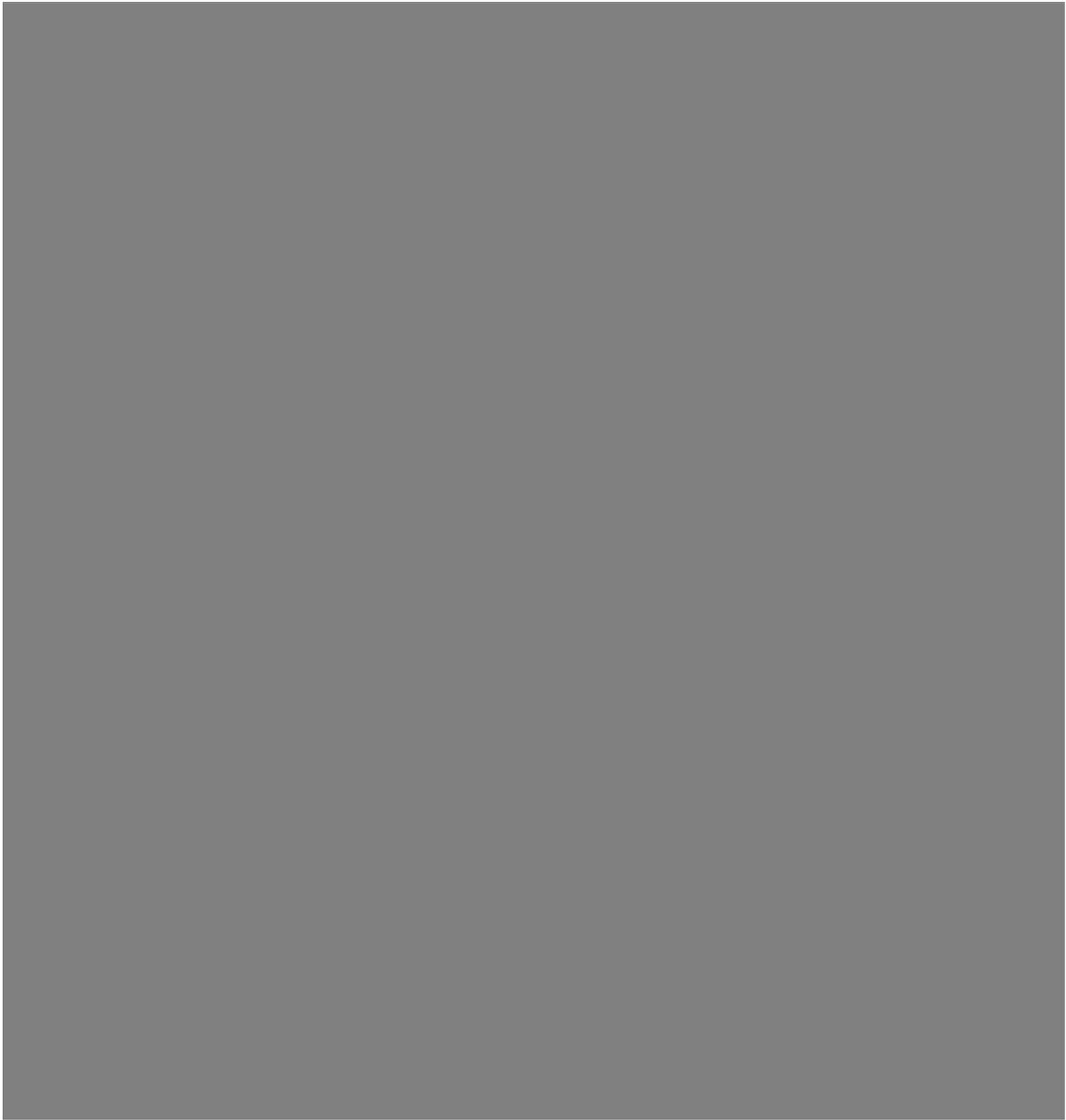
## Appendix 6.2

### General description of methods











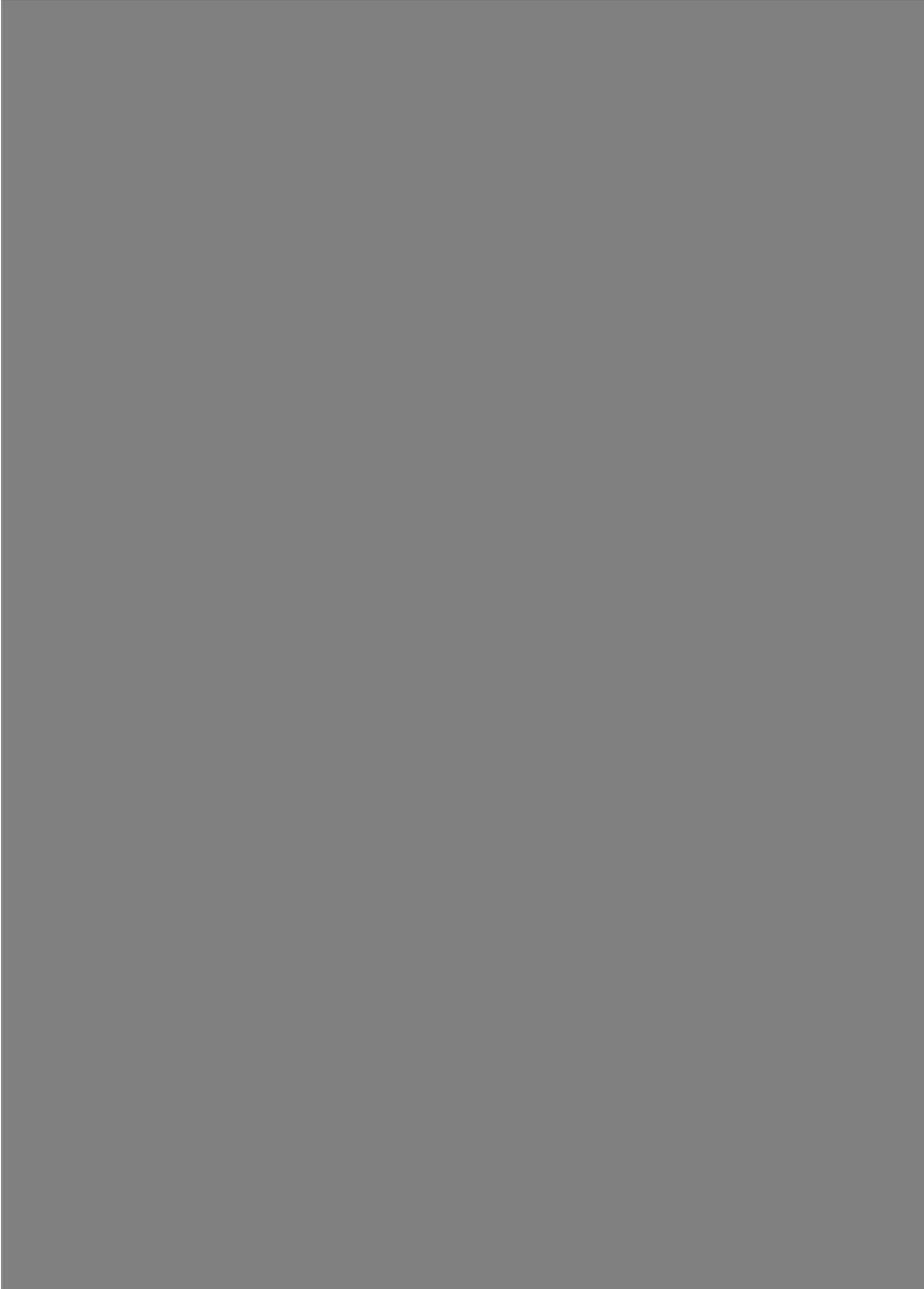


## Appendix 6.3

### Characterization of the *amyL* locus in the production strain

To verify that the modification had been introduced in *amyL* as intended, the DNA sequence of the locus in the production strain was determined as described under General methods (Appendix 6.2). The overview of the *amyL* locus and the annotated sequence in the production strain can be found below.

Annotated sequence of the *amyL* locus in the production strain:







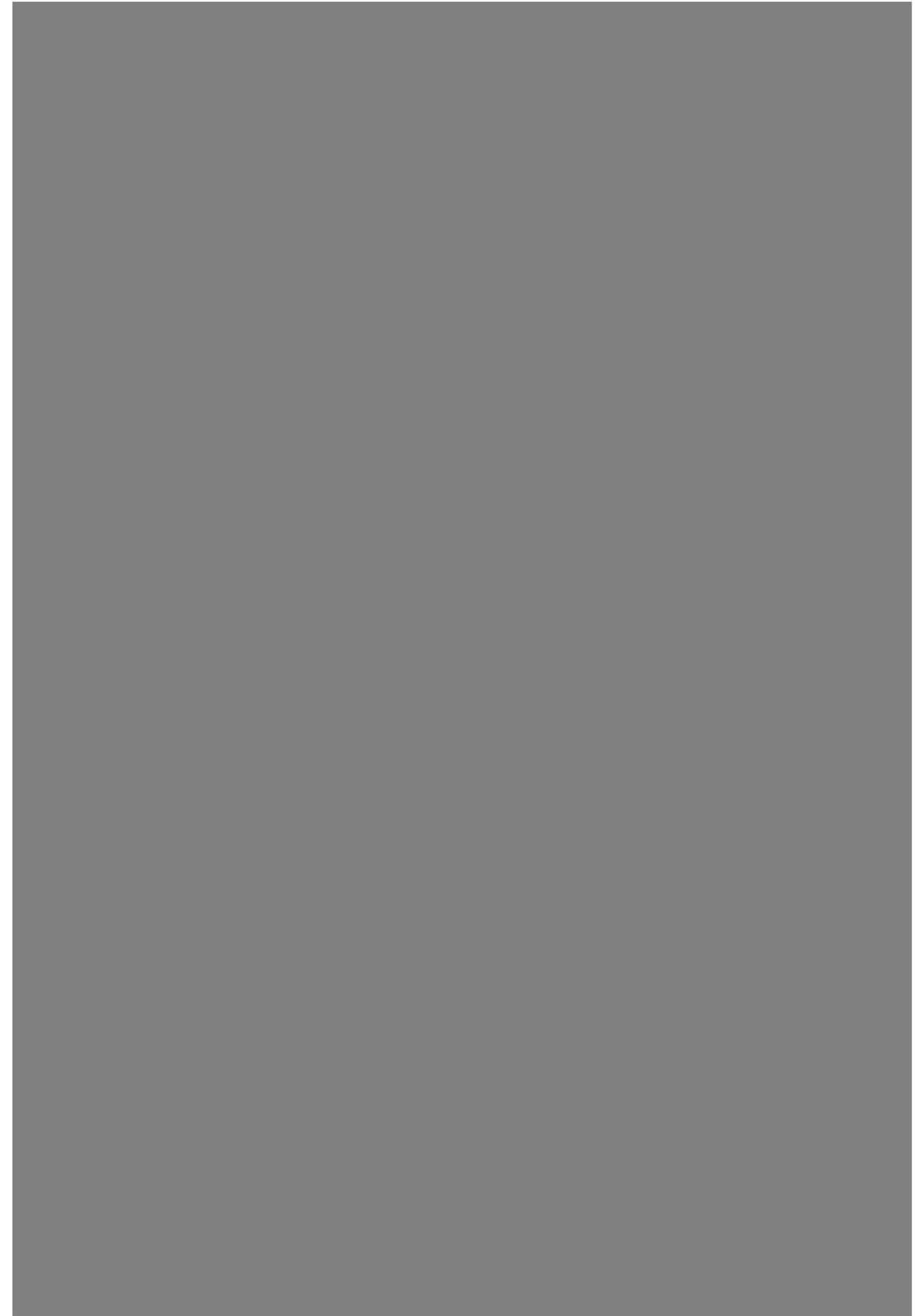


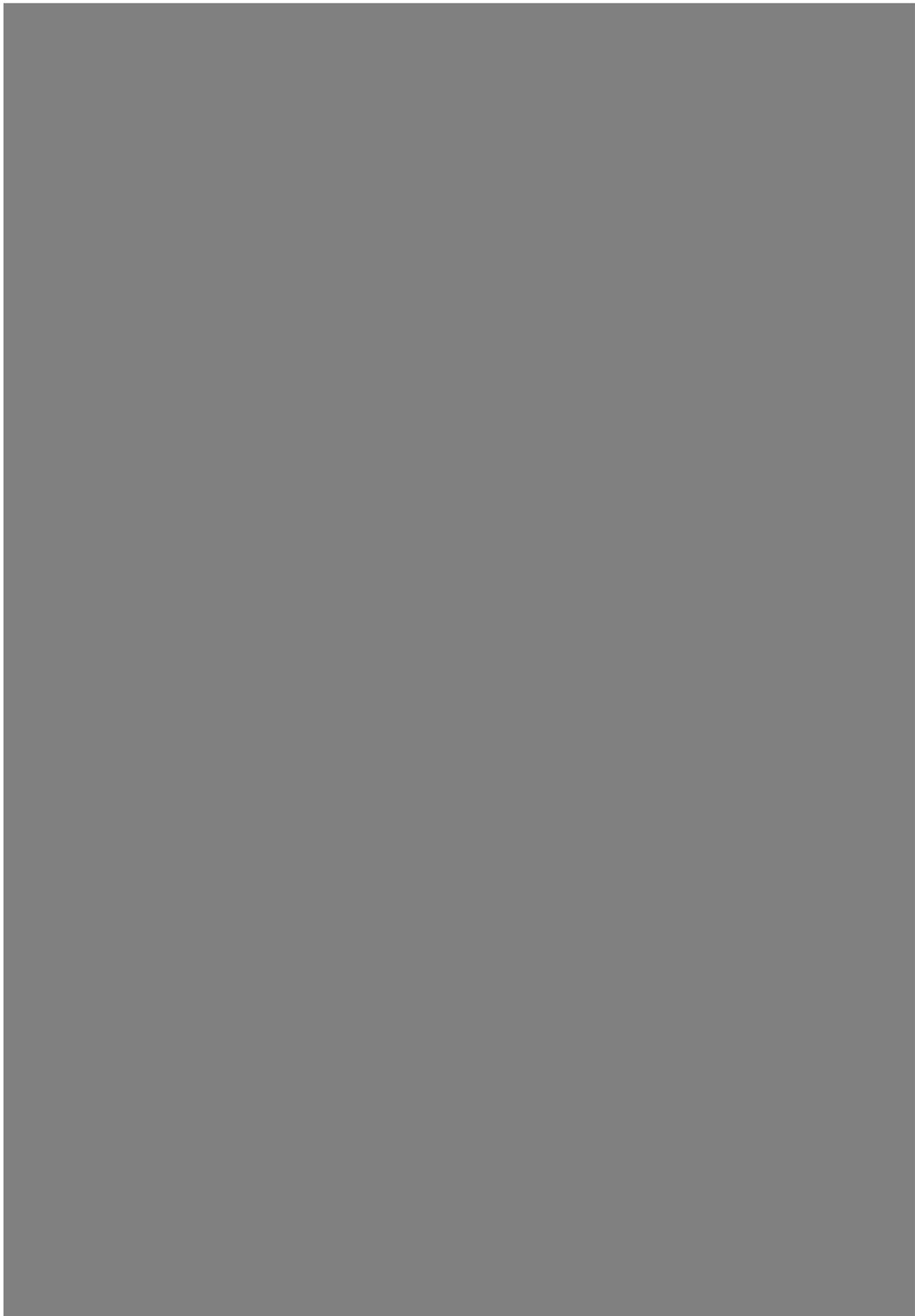
## Appendix 6.4

### Characterization of the *xyIA* locus in the production strain

To verify that the modification had been introduced in *xyIA* as intended, the DNA sequence of the locus in the production strain was determined as described in the general methods (Appendix 6.2). The overview of the *xyIA* locus and the annotated sequence in the production strain can be found below.











## Appendix 6.5

### Genetic stability of the production strain [REDACTED]

#### Summary

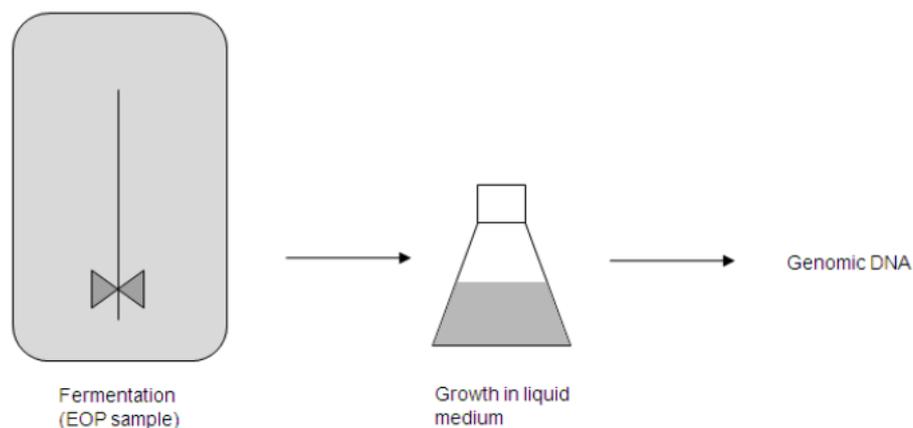
The genetic stability of the production strain was analysed by Southern blot analysis on genomic DNA obtained from end of production samples and compared to a reference of genomic DNA from the production strain taken from the vial collection.

The Southern blot analysis of the end of production samples and the reference sample showed no differences in the band pattern, thereby demonstrating the genetic stability of the inserted DNA in the serine protease production strain.

#### Details

The genetic stability of the serine protease production strain was analysed by Southern blot analysis on genomic DNA obtained from end of production samples from three independent batches (RHF24, RHF25 and RHF26).

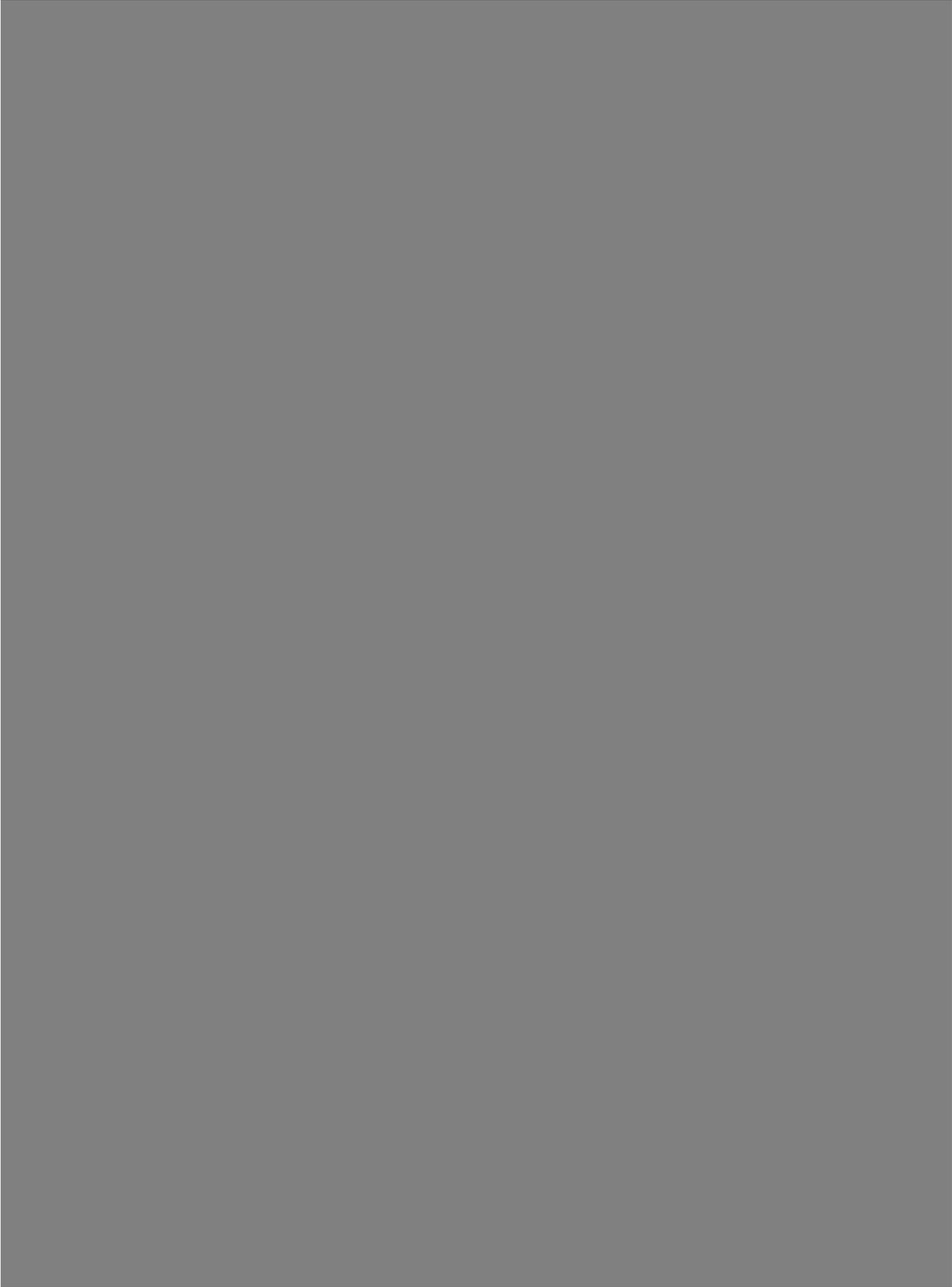
Genomic DNA was isolated from culture suspension (*i.e.*, end of production samples) that were allowed to grow in liquid culture (Fig. 1). This propagation step adds additional generations to the cells used for the analysis allowing the analysis of genetic stability over the intended period of production.



**Fig. 1:** Overview of genomic DNA sample preparation for genetic stability analysis. A sample from end of production (EOP) from each lot batch was taken and used to inoculate liquid medium to allow for growth of the strain for 1 day. The bacterial cells were harvested and genomic DNA was extracted for Southern blot analysis.

The DNA derived from the end of production samples (Fig. 1) was subsequently analysed by Southern blot analysis, comparing to DNA of the production strain.

End of production (EOP) samples from the independent production batches were analyzed. Hybridisation to a gene-specific probe [REDACTED] (Fig. 2) resulted in 2 bands derived from the two copies of the [REDACTED] construct inserted in the production strain. The Southern blot bands from three representative batches correspond to the bands detected in the production strain (Fig. 2). The band sizes are in agreement with the expected sizes listed in Table 1.



The presented results verify the presence of 2 copies of the [REDACTED] tandem gene construct (i.e., a total of 4 gene copies of [REDACTED], one copy present at each of the targeted loci, *amyL* and *xyIA*). Southern blot analysis showed identical band pattern between the production strain and the samples derived from end of production. Thus, it is concluded that the production strain is genetically stable.

#### **Method for Southern blot analysis**

Genomic DNA was purified from the samples and [REDACTED]. DNA fragments were separated on an 0.75% agarose gel and transferred to a nylon membrane. Hybridization was performed using a [REDACTED]

[REDACTED] Fig. 2).