



**Comparison of Phosphinothricin Acetyltransferase (PAT) Protein
Produced in Recombinant *Escherichia coli* and PAT Protein
Produced in Event SYHT0H2 Derived Soybean Plants**

Final Report

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STATEMENTS OF DATA CONFIDENTIALITY CLAIMS

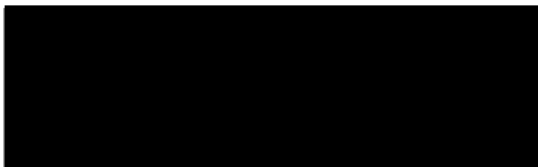
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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The portion of this study conducted in the United States was conducted in accordance with the United States Environmental Protection Agency Federal Insecticide, Fungicide, and Rodenticide Good Laboratory Practice Standards (40 CFR Part 160, US EPA 1989) with the following exceptions:

- Planting and cultivation of the seed test and control substances in the greenhouse were not conducted according to GLP standards
- Plant tissue was collected before the initiation of this study
- The purchased standards were characterized by the manufacturer prior to use in this study
- The seed control substance (10SG900137) was not characterized according to GLP standards.

The peptide mass mapping and N-terminal analysis portions of this study were conducted in accordance with the Organization for Economic Co-operation and Development GLP Standards.

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QUALITY ASSURANCE STATEMENT

Study Title: Comparison of Phosphinothricin Acetyltransferase (PAT) Protein Produced in Recombinant *Escherichia coli* and PAT Protein Produced in Event SYHT0H2 Derived Soybean Plants

Study Director: [REDACTED]

Study Number: TK0059680

Pursuant to Good Laboratory Practice Regulations (40 CFR Part 160), this statement verifies that the aforementioned study was inspected and/or audited and the findings reported to Management and to the Study Director by the Quality Assurance Unit on the dates listed below.

<u>Inspection/Audit Type</u>	<u>Inspection/Audit Dates</u>	<u>Reporting Date</u>
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Please reference the analytical phase report for additional audit/inspection dates.

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GENERAL INFORMATION

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The following contributed to this report in the capacities indicated:

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The peptide mass mapping analysis and the N-terminal amino acid sequence analysis were conducted by SGS M-Scan Ltd., 3 Millars Business Centre, Fishponds Close, Wokingham, Berkshire, RG41 2TZ, UK. Additional personnel associated with these analyses are listed in Appendix B.

Study Dates

Study initiation date:	February 1, 2012
Experimental start date:	February 8, 2012
Experimental end date:	May 23, 2012

Records Retention

Raw data, the original copy of this report, and other relevant records are archived at Syngenta, 3054 East Cornwallis Road, Research Triangle Park, NC 27709-2257, USA. Facility records for SGS M-Scan Ltd. are archived SGS M-Scan Ltd., 3 Millars Business Centre, Fishponds Close, Wokingham, Berkshire, RG41 2TZ, UK.

TABLE OF CONTENTS

STATEMENTS OF DATA CONFIDENTIALITY CLAIMS	2
GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT	3
QUALITY ASSURANCE STATEMENT	4
GENERAL INFORMATION	5
TABLE OF CONTENTS	6
LIST OF TABLES	8
LIST OF FIGURES	8
LIST OF ACRONYMS AND ABBREVIATIONS	9
1.0 EXECUTIVE SUMMARY	10
2.0 INTRODUCTION	11
3.0 MATERIALS AND METHODS	11
3.1 Microbially Produced PAT	11
3.2 Seed Test and Control Substances	12
3.3 Preparation of Plant-produced PAT and Nontransgenic Plant Controls	12
3.3.1 Extracts of Event SYHT0H2 soybean seed and nontransgenic control soybean seed	12
3.3.2 Purified PAT preparation from SYHT0H2 extract	13
3.3.3 Microbially produced PAT-fortified nontransgenic extract	13
3.4 PAT Protein Quantification	14
3.5 Total Protein Determination	14
3.6 Immunoreactivity and Molecular Weight Determination	14
3.7 Specific Enzymatic Activity	15
3.7.1 Enzymatic activity assay	15
3.7.2 Calculation of enzymatic activity	16
3.8 Glycosylation Analysis	16
3.9 Peptide Mass Mapping Analysis	17
3.10 N-Terminal Amino Acid Sequence Analysis	17
3.11 Control of Bias	17
3.12 Statistical Methods	17
4.0 RESULTS AND DISCUSSION	18
4.1 Immunoreactivity and Molecular Weight	18
4.2 Specific Enzymatic Activity	20
4.3 Glycosylation Analysis	20
4.4 Peptide Mass Mapping	22

4.5	N-Terminal Amino Acid Sequence Analysis.....	23
4.6	Data Quality and Integrity.....	23
5.0	CONCLUSIONS	23
6.0	REFERENCES	24
	APPENDICES SECTION	26
APPENDIX A	Enzymatic Activity Assay LOD Determination	27
APPENDIX B	Peptide Mass Mapping and N-terminal Sequencing Phase Report.....	29

LIST OF TABLES

TABLE 1	Seed test and control substances	12
TABLE 2	Protein preparations and use in subsequent analyses	13
TABLE 3	Specific enzymatic activity of the microbially produced and plant-produced PAT	20

LIST OF FIGURES

FIGURE 1	Reaction catalyzed by PAT	15
FIGURE 2	Western blot analysis of plant-produced PAT, nontransgenic soybean seed extract fortified with microbially produced PAT, nontransgenic soybean seed extract, PAT purified from SYHT0H2 soybean seed extract, and the microbially produced PAT	19
FIGURE 3	Glycosylation analysis of a PAT purified preparation from SYHT0H2 soybean seed extract, and the microbially produced PAT	21
FIGURE 4	Amino acid sequence identified by peptide mass mapping analysis of the microbially produced PAT (Moens 2012)	22
FIGURE 5	Amino acid sequence identified by peptide mass mapping analysis of a PAT purified preparation from SYHT0H2 soybean seed extract	22

LIST OF ACRONYMS AND ABBREVIATIONS

Definitions of International System of Units (SI) base units and derived units may be found in NIST (2011).

Acetyl CoA	acetyl coenzyme A
AvHPPD-03	<i>p</i> -hydroxyphenylpyruvate dioxygenase enzyme encoded by <i>avhppd-03</i>
BCA	bicinchoninic acid
BCIP	5-bromo-4-chloro-3-indolyl phosphate
Bis-Tris	bis(2-hydroxyethyl)imino-tris(hydroxymethyl)methane
BSA	bovine serum albumin
CoASH	coenzyme A
DTNB	5,5'-dithiobis(2-nitrobenzoic acid)
ECL™	enhanced chemiluminescence
EDTA	ethylenediaminetetraacetate
ELISA	enzyme-linked immunosorbent assay
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GLP	Good Laboratory Practices
HGA	homogentisic acid or homogentisate
HPLC	high performance liquid chromatography
HPPD	<i>p</i> -hydroxyphenylpyruvate dioxygenase enzyme
kDa	kiloDalton
LC-MS/MS	liquid chromatography – mass spectrometry (tandem mass spectrometry)
LDS	lithium dodecylsulfate
LOD	limit of determination
MES	2-(<i>N</i> -morpholino)ethanesulfonic acid
NBT	p-nitro blue tetrazolium chloride
OD	optical density
PAT	phosphinothricin acetyltransferase enzyme encoded by <i>pat</i>
PVDF	polyvinylidene difluoride
Q-TOF	quadrupole time-of-flight
RSD	relative standard deviation
RT-PCR	real-time polymerase chain reaction
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SQC	Stewardship Quality Control
TNB ²⁻	2-nitro-5-thiobenzoate anion
Tris	tris(hydroxymethyl)aminomethane
Tween 20	polyoxyethylene sorbitan monolaurate
U	unit
US EPA	United State Environmental Protection Agency
v/v	volume/volume
§	section

1.0 EXECUTIVE SUMMARY

In order to assess the biochemical and functional equivalence between the phosphinothricin acetyltransferase (PAT) protein produced in a recombinant *Escherichia coli* expression system and the PAT protein produced in soybean derived from Event SYHT0H2, the proteins from both sources were compared with respect to identity, integrity, specific enzymatic activity, and glycosylation status.

Western blot analysis of the microbially produced and plant-produced PAT proteins showed identical mobility consistent with the predicted molecular weight of 20.6 kDa. Additionally, the microbially produced and plant-produced PAT cross-reacted with the same PAT-specific antibody, confirming similar immunoreactivity for both proteins. A specific enzymatic activity assay revealed that the microbially produced and plant-produced PAT were comparable in their specific activity. The microbially produced PAT was assessed in the presence of nontransgenic, near-isogenic soybean seed extract to simulate the experimental extraction conditions of the plant-produced PAT. The specific activity was 22.13 Units/mg PAT and 30.58 Units/mg PAT for the microbially produced PAT and the plant-produced PAT, respectively. There was no evidence of post-translational glycosylation of the microbially produced or plant-produced PAT. A previous study identified 76.5% of the predicted amino acid sequence of the microbially produced protein by peptide mass mapping. In this study, peptide mass mapping yielded coverage of 63.4% of the predicted amino acid sequence of the plant-produced PAT, confirming the identity of the protein from both sources. Prior to this study, N-terminal sequencing results confirmed the intact N-terminus of the microbially produced PAT protein. In this study, except for the cleavage of the first amino acid (methionine) from the N-terminus of the plant-produced protein, the N-terminal peptide of PAT was consistent with the predicted sequence, confirming the identity of the protein from both sources.

The results of this study demonstrate that the microbially produced PAT is biochemically and functionally equivalent to PAT produced in SYHT0H2 soybean and supports the conclusion that the microbially produced PAT is a suitable surrogate to evaluate the safety of PAT produced in SYHT0H2 soybean.

2.0 INTRODUCTION

The purpose of this study was to assess the biochemical and functional equivalence between microbially produced phosphinothricin acetyltransferase (PAT) protein and PAT produced in transgenic soybean plants derived from Event SYHT0H2.

Soybean (*Glycine max* [L.] Merrill) has been genetically modified to express the genes *avhppd-03* derived from oat (*Avena sativa* L.) and *pat* derived from *Streptomyces viridochromogenes*. The gene *avhppd-03* encodes a *p*-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme, designated AvHPPD-03, that catalyzes the formation of homogentisic acid (HGA), the aromatic precursor in plastoquinone and vitamin E biosynthesis. In comparison with the native soybean HPPD, the AvHPPD-03 isozyme from oat has lower binding affinity for mesotrione, an herbicide that inhibits HPPD. Expression of *avhppd-03* in the transgenic Event SYHT0H2 soybean plants confers a mesotrione-tolerance phenotype. The gene *pat* encodes the enzyme phosphinothricin acetyltransferase (PAT), which inactivates the herbicide glufosinate, an inhibitor of glutamine synthetase, an enzyme in the nitrogen assimilation pathway. Expression of *pat* confers a glufosinate-tolerance phenotype, which was used as a selectable marker in the development of Event SYHT0H2 soybeans.

In this study, key biochemical and functional parameters were evaluated to assess whether the microbially produced PAT is a suitable surrogate for PAT produced in SYHT0H2 soybean. Microbially produced and plant-produced PAT were compared with respect to identity, integrity, specific enzymatic activity, and glycosylation status. Establishing functional and biochemical equivalence supports the use of microbially produced PAT in studies evaluating the safety of PAT in SYHT0H2 soybean.

3.0 MATERIALS AND METHODS

3.1 Microbially Produced PAT

Microbially produced PAT was prepared by Bayer CropScience in 1995 from a recombinant *Escherichia coli* expression system. The genes expressed in the microbial system and in SYHT0H2 soybean encode proteins identical in amino acid sequence.

Prior to this study, microbially produced PAT was prepared from *E. coli* cell paste by Bayer CropScience in 1995 (Ghent, Belgium), and further purified and lyophilized into a powder. The resulting dry formulation was designated protein batch #1995, and used in a number of studies supporting the safety of the PAT protein. An aliquot of the test substance was shipped on dry ice to Syngenta Crop Protection, LLC (Research Triangle Park, NC, USA) and stored at -80°C ±10°C until further use.

Protein batch #1995 was determined to contain 78.3 % PAT by weight; the molecular weight of PAT was consistent with the predicted molecular weight of 20.6 kDa (Dreesen 2011). The microbially produced PAT was resolubilized in 10 mM tris(hydroxymethyl)aminomethane (Tris), 0.1% (v/v) Tween® 20, 0.4 mM ethylenediaminetetraacetate (EDTA), pH 8.9, and included in Western blot, specific enzymatic activity, and glycosylation status analyses.

3.2 Seed Test and Control Substances

The seed test substance for this study was soybean seed lot 10SG900903 (SYHT0H2 soybean). The control substance was nontransgenic, near-isogenic soybean seed in the same genetic background as the seed test substance. Table 1 shows the descriptions and pedigree codes for the seed test and control substances.

TABLE 1 Seed test and control substances

Seed identification	Material identification	Pedigree code
SYHT0H2 soybean (test)	10SG900903	SYHT0H2
Nontransgenic (control)	10SG900137	JACK

The seed test substance was characterized by real-time polymerase chain reaction (RT-PCR) analysis (Ingham *et al.* 2001) to confirm identity and purity (Burgin 2011). Identification of the nontransgenic, near-isogenic soybean seed was also verified by RT-PCR for Stewardship Quality Control (SQC) testing according to the current Syngenta standards.

3.3 Preparation of Plant-produced PAT and Nontransgenic Plant Controls

Prior to this study, the seed test and control substances (Table 1) were generated under standard greenhouse conditions. Seed from SYHT0H2 soybean and the nontransgenic, near isogenic control soybean were collected, ground into a fine powder, and stored at 2-8°C.

3.3.1 Extracts of Event SYHT0H2 soybean seed and nontransgenic control soybean seed

Protein for use in Western blot analysis and enzymatic activity assays was extracted from the SYHT0H2 soybean powder by resuspending it in 100 mM borate buffer (pH 7.5) containing 0.2% (v/v) polyvinylpyrrolidone, 7.7 mM sodium azide, 0.5% (v/v) Tween 20® surfactant, 1.2% (v/v) hydrochloric acid, and supplemented with protease inhibitor cocktail (Roche, 1 tablet/50 ml). The mixture was homogenized and incubated on ice. After incubation on ice, the extract was centrifuged and filtered through a 0.22 µm Millipore filter unit; the resulting extract was designated SYHT0H2 extract. The concentration of PAT and total protein were determined by enzyme-linked immunosorbent assay (ELISA) and the bicinchoninic acid (BCA) protein assay, respectively.

Nontransgenic, near-isogenic control soybean powder was extracted in parallel with the preparation of the SYHT0H2 extract, employing the same method. The resulting preparation was designated as the nontransgenic extract. The nontransgenic extract was analyzed by the BCA assay to determine the concentration of total protein in the sample. The nontransgenic extract was used as a negative control in the Western blot and enzymatic activity assays (Table 2).

3.3.2 Purified PAT preparation from SYHT0H2 extract

SYHT0H2 extract was prepared as described in §3.3.1 and the PAT protein was immunopurified from the SYHT0H2 seed extract. An immunoaffinity column, prepared with monoclonal anti-PAT antibody, was used to purify the PAT from the SYHT0H2 extract. The SYHT0H2 extract was applied to the equilibrated immunoaffinity column, the column was washed to remove unbound proteins, and PAT was eluted in 100 mM glycine buffer (pH 2.5) and neutralized. The purified protein was dialyzed into 0.1 M sodium bicarbonate, pH 8.1, and concentrated by ultrafiltration. The resulting sample was analyzed by ELISA to determine the concentration of PAT, and stored at $-20^{\circ}\text{C} \pm 8^{\circ}\text{C}$ in lithium dodecyl sulfate (LDS) sample buffer until further use. The purified PAT preparation from SYHT0H2 extract was used in Western blot, glycosylation status, N-terminal sequencing, and peptide mass mapping analyses (Table 2).

3.3.3 Microbially produced PAT-fortified nontransgenic extract

Nontransgenic soybean seed extract was prepared as described in §3.3.1 and fortified with microbially produced PAT. This sample was analyzed by Western blot and enzymatic activity assays in order to investigate if the plant matrix had an effect on PAT enzymatic activity, mobility or immunoreactivity (Table 2). Inclusion of this sample allowed for the comparison of the microbially produced and plant-produced PAT in the same matrix.

TABLE 2 Protein preparations and use in subsequent analyses

Analysis	Samples included in the analysis	Purpose of the analysis
Western blot	<ul style="list-style-type: none">• microbially produced PAT• SYHT0H2 extract• purified preparation from SYHT0H2 extract• nontransgenic extract• nontransgenic extract fortified with microbially produced PAT	Examine PAT apparent molecular weight, intactness, and relative immunoreactivity
Specific enzymatic activity	<ul style="list-style-type: none">• microbially produced PAT• SYHT0H2 extract• nontransgenic extract• nontransgenic extract fortified with microbially produced PAT	Confirm functional equivalence of both proteins. Confirm correct folding of both proteins.
Glycosylation	<ul style="list-style-type: none">• microbially produced PAT• purified preparation from SYHT0H2 extract	Confirm the absence of glycosyl residues
Peptide mass mapping	<ul style="list-style-type: none">• purified preparation from SYHT0H2 extract	Confirm the identity of plant-produced PAT
N-terminal sequencing	<ul style="list-style-type: none">• purified preparation from SYHT0H2 extract	Confirm the N-terminal amino acid sequence of plant-produced PAT

3.4 PAT Protein Quantification

The concentration of PAT was determined by ELISA (Tijssen 1985) prior to conducting Western blot, glycosylation, peptide mass mapping, and N-terminal sequencing analyses. PAT quantification was performed using the Envirologix Qualiplate™ ELISA Kit for PAT. PAT was captured on ELISA plates pre-coated with the capture antibody. An antibody-enzyme conjugate was used to bind the PAT protein and detection was accomplished through conversion of a colorimetric substrate. The concentration of PAT was proportional to the measured absorbance values. Samples were then quantified relative to a standard curve of known PAT concentrations. Samples and standards were applied to the microtiter plate in triplicate. The absorbance values were measured with a spectrophotometer at dual wavelengths of 450 and 650 nm.

The results were analyzed with Molecular Devices SoftMax Pro® GxP software, version 5.4.1, using a four-parameter algorithm. For each sample, the mean concentration of dilutions within the quantitative range of the ELISA was calculated.

3.5 Total Protein Determination

Total protein was quantified *via* the BCA method (Hill and Straka 1988), using bovine serum albumin (BSA) as the reference protein standard. The results were analyzed with Molecular Devices SoftMax Pro® GxP software, version 5.4.1, using a four-parameter algorithm. For each sample, the mean concentration of all dilutions within the quantitative range of the BCA assay was calculated.

3.6 Immunoreactivity and Molecular Weight Determination

Western blot analysis was used to investigate the identity and integrity of PAT from 1) the microbially produced PAT, 2) SYHT0H2 soybean seed extract, 3) nontransgenic extract fortified with microbially produced PAT, and 4) a purified preparation from SYHT0H2 soybean seed extract. Based on ELISA results, aliquots containing 10 ng of PAT prepared in lithium dodecylsulfate sample buffer were subjected to sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions using a 12% Bis-Tris gel and 2-(*N*-morpholino)ethanesulfonic acid (MES) running buffer. Based on BCA analysis, an aliquot of the nontransgenic, near-isogenic seed extract, with total protein equivalent to the plant-produced PAT sample prepared for Western blot, was included in the analysis as a negative control. The molecular weight standard was SeeBlue® Plus2 pre-stained standard.

The protein was transferred to a polyvinylidene fluoride (PVDF) membrane via electroblotting. The membrane was probed with a polyclonal goat antibody capable of detecting PAT. Detection of PAT was accomplished by binding of a polyclonal donkey anti-goat immunoglobulin G antibody conjugated with alkaline phosphatase enzyme, which catalyzes the conversion of the colorimetric substrate BCIP/NBT. The Western blot was visually examined for the presence of intact immunoreactive PAT or other immunoreactive PAT-derived fragments.

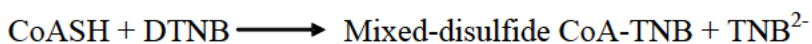
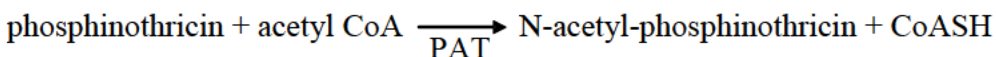
3.7 Specific Enzymatic Activity

3.7.1 Enzymatic activity assay

The specific enzymatic activity of PAT from 1) the microbially produced PAT, 2) SYHT0H2 soybean seed extract, and 3) nontransgenic extract fortified with microbially produced PAT, was measured in triplicate analyses using a continuous spectrophotometric assay based on the method described by Thompson *et al.* (1987) and D'Halluin *et al.* (1992). Nontransgenic soybean seed extract was included within the enzymatic activity assay as a negative control sample.

As shown in the reaction diagram below, PAT catalyzes the transfer of the acetyl group from acetyl coenzyme A (acetyl CoA) to phosphinothricin. The released free thiol (CoASH) reacts with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) to form 2-nitro-5-thiobenzoate anion (TNB²⁻) under mild alkaline conditions (pH 7-8) (Habeeb 1972). The molar formation of TNB²⁻ can be monitored by measuring absorbance increase at 412 nm and can be directly converted into the molar acetylation of phosphinothricin by PAT. The enzymatic activity for PAT is reported as U/mg PAT where one unit (U) of PAT activity is defined as the amount of enzyme required to acetylate 1 μ mol of phosphinothricin per minute (equivalent to 1 μ mol DTNB reduced or 1 μ mol TNB²⁻ produced per minute) under the described reaction conditions.

FIGURE 1 Reaction catalyzed by PAT



PAT = Phosphinothricin acetyltransferase
Acetyl CoA = acetyl coenzyme A
CoASH = coenzyme A
DTNB = 5,5'-dithiobis(2-nitrobenzoic acid)
TNB²⁻ = 2-nitro-5-thiobenzoate anion

The enzymatic activity assay for PAT, in which the production of TNB²⁻ is measured using a continuous spectrophotometric assay, was validated as suitable for the purpose of quantifying PAT activity (Song 2011). Specific enzymatic activity values differing by $\leq 30\%$ are considered comparable, and support a conclusion of functional equivalence.

The enzymatic reactions were conducted in 96-well plates. Following a 2-minute preincubation at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$, the reaction was initiated by adding PAT to an assay mixture containing 1 mM phosphinothricin, 1 mM acetyl CoA and 1 mM DTNB in 50 mM Tris, 2 mM EDTA, and 0.5 mg/ml BSA, pH 7.5. The total volume of the reaction mixture was 100 μ l.

The formation of TNB²⁻ was monitored spectrophotometrically using Molecular Devices SoftMax Pro® GxP software, version 5.4.1 at 412 nm at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ over 5 minutes, with readings taken every 12 seconds. The extinction coefficient of TNB²⁻ (Riddles *et al.* 1979, Riddles *et al.* 1983) is $14,150 \text{ M}^{-1} \text{ cm}^{-1}$, and was used for calculating the amount of TNB²⁻ formed in accordance with the Beer-Lambert law (Aitken and Learmonth 1996).

3.7.2 Calculation of enzymatic activity

The concentration of TNB^{2-} was calculated in accordance with the Beer-Lambert law as follows:

$$A_{412} = \epsilon \times c \times l$$

A_{412} = absorbance measurement at 412 nm
 ϵ = extinction coefficient of TNB^{2-}
 c = concentration
 l = optical pathlength

The reaction rate (milli OD/min) was determined by plotting A_{412} vs. time, and is represented by the slope of the trendline. The slope was calculated by the Softmax Pro® GxP software, version 5.4.1, using a moving range, and was determined over 10 consecutive timepoints throughout the 5 minute timecourse. The highest reaction rate was corrected for the pathlength of each reaction well, and is reported as the V_{max} (milli OD/min*cm).

The slope of the trendline was calculated:

$$y = mx + b$$

m = slope
 b = y-intercept

Using the V_{max} and the amount of PAT in the reaction as determined by ELISA, the specific enzymatic activity was calculated:

$$\text{Specific Activity } \left(\frac{\text{U}}{\text{mg PAT}} \right) = \frac{\left(\frac{V_{\text{max}} \left(\frac{\text{milli OD}}{\text{min} * \text{cm}} \right) \times 10^{-3} \left(\frac{\text{OD}}{\text{milli OD}} \right)}{\epsilon \left(\frac{\text{L}}{\text{mol TNB}^{2-} * \text{cm}} \right) \left(\frac{\text{ml}}{\text{mmol}} \right) \times 10^{-3} \left(\frac{\text{mmol}}{\mu\text{mol}} \right)} \times \text{reaction volume (ml)} \right)}{\text{mg PAT}}$$

OD = optical density (absorbance measurement at 412 nm)

A mean specific activity value was calculated from triplicate activity assays for all samples.

3.8 Glycosylation Analysis

The microbially produced PAT and PAT purified preparation from SYHT0H2 soybean seed extract were analyzed with the ECL™ Glycoprotein Detection Module Kit to confirm the absence of glycosyl residues. Samples were subjected to SDS-PAGE under reducing conditions using a 12% Bis-Tris gel and MES running buffer. Aliquots containing approximately 1000 and 500 ng of a PAT purified preparation from SYHT0H2 soybean seed extract, and 1000 ng of the microbially produced PAT, were applied to the gel. Transferrin, a glycosylated protein, was applied to the gel at 10, 25, 50, and 100 ng as a positive control. Soybean trypsin inhibitor, a nonglycosylated protein, was applied on the gel at 1000 ng as a negative control. The molecular weight standard was SeeBlue® Plus2 pre-stained standard.

The PAT protein was electroblotted onto a PVDF membrane. While on the membrane, glycan moieties were oxidized using sodium metaperiodate, labeled with biotin, and detected with alkaline-phosphatase-linked streptavidin.

3.9 Peptide Mass Mapping Analysis

Aliquots containing approximately 0.75 µg of a PAT purified preparation from SYHT0H2 soybean seed extract were subjected to SDS-PAGE under reducing conditions using a 12% Bis-Tris gel and MES running buffer. The gel was stained with Coomassie® blue, and sent to SGS M-Scan Ltd. (Wokingham, UK).

The protein bands, consistent with the predicted molecular weight of PAT, were excised from the gel. The protein was reduced, alkylated with iodoacetamide, and enzymatically digested using trypsin, chymotrypsin, and flavastacin (Asp-N). A separate digestion was conducted with each enzyme. The digested samples were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a quadrupole time-of-flight mass spectrometer (Q-TOF Xevo, Waters) coupled to a nano liquid chromatography instrument (Ultimate 3000 nano-HPLC, Dionex). The detected peptide masses were searched using MASCOT® Software against a protein database containing the expected amino acid sequence of PAT (Appendix B). Peptide mass mapping analysis was conducted by SGS M-Scan Ltd. (Wokingham, UK).

3.10 N-Terminal Amino Acid Sequence Analysis

The N-terminal amino acid sequence of a PAT purified preparation from SYHT0H2 soybean seed extract was determined and compared with the predicted amino acid sequence. Aliquots containing approximately 0.75 µg of the PAT protein were subjected to SDS-PAGE under reducing conditions using a 12% Bis-Tris gel and MES running buffer, followed by electroblotting to a PVDF membrane. The blot was stained with amido black, and the protein bands corresponding to the predicted molecular weight of PAT were excised, and sent to SGS M-Scan Ltd. (Wokingham, UK).

The samples were applied to an automated pulsed-liquid sequencer for N-terminal amino acid sequence analysis. The methodology used was developed for proteins immobilized on a PVDF membrane and optimized for automated Edman degradation analysis (Appendix B). The N-terminal amino acid sequencing was conducted by SGS M-Scan Ltd. (Wokingham, UK).

3.11 Control of Bias

Any rejected data, and the documented reasons for the rejection of those data, will be retained in the study file.

3.12 Statistical Methods

Means and relative standard deviations were calculated using Microsoft Office Excel® 2007 software.

4.0 RESULTS AND DISCUSSION

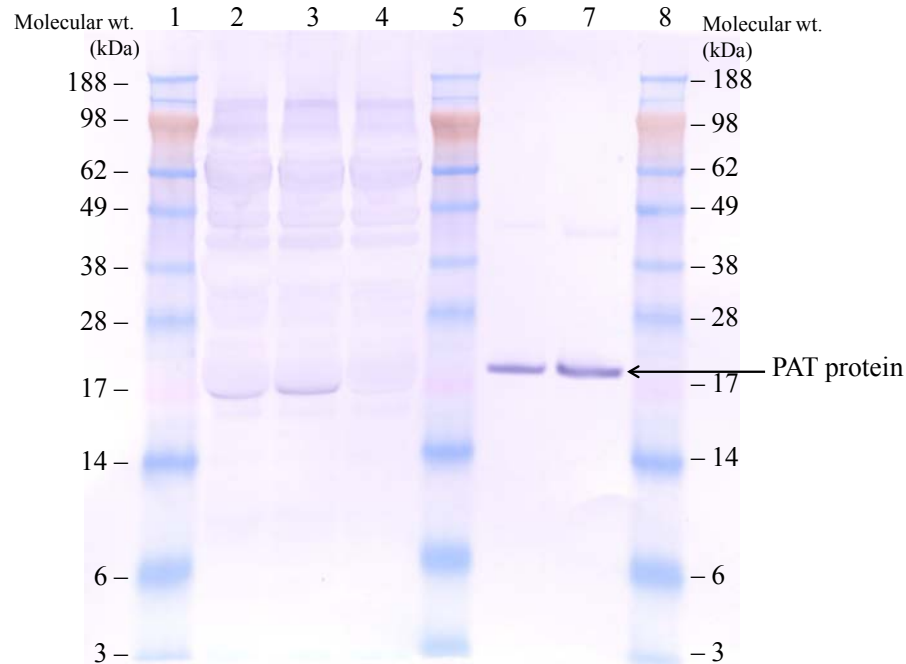
4.1 Immunoreactivity and Molecular Weight

Western blot analysis of the microbially produced and PAT purified preparation from SYHT0H2 soybean seed extract revealed a single and sharp immunoreactive band consistent with the predicted molecular weight of the PAT protein (Figure 2, Lanes 6 and 7) confirming the identity and integrity of PAT in both sources.

Western blot analysis of the microbially produced and PAT purified preparation from SYHT0H2 soybean seed extract also revealed a faint protein band with a molecular weight of approximately 43 kDa (Figure 2, Lanes 6 and 7). Since this protein cross-reacted with a PAT-specific antibody, and its apparent molecular weight is consistent with that of two PAT molecules, it most likely represents a dimer of PAT.

The crude SYHT0H2 extract, which contained PAT in the presence of the plant matrix, revealed a faint immunoreactive band with higher mobility (i.e., lower apparent molecular weight) compared to the intensity and mobility of the PAT protein in the microbially produced test substance and a PAT purified preparation from SYHT0H2 soybean seed extract (Figure 2, Lane 2). However, similar intensity and mobility of the PAT protein is observed in both the crude SYHT0H2 extract and nontransgenic extract fortified with microbially produced PAT (Figure 2, Lanes 2 and 3), indicating that the diminished intensity and higher mobility of the PAT protein in these samples is most likely a result of matrix interference, due to the high concentration of endogenous seed storage proteins in the crude soybean seed extract. Additional faint immunoreactive bands observed in the crude SYHT0H2 extract, between 17 kDa and 188 kDa molecular weight, are also observed in the nontransgenic, near-isogenic seed extract, indicating that these bands are most likely due to nonspecific binding with endogenous proteins contained in the matrix (Figure 2, Lanes 2-4).

FIGURE 2 Western blot analysis of plant-produced PAT, nontransgenic soybean seed extract fortified with microbially produced PAT, nontransgenic soybean seed extract, PAT purified from SYHT0H2 soybean seed extract, and the microbially produced PAT



Lane 1: Molecular weight standard

Lane 2: Crude SYHT0H2 soybean seed extract (10 ng PAT, 64.52 µg total protein)

Lane 3: Nontransgenic soybean seed extract fortified with microbially produced PAT (10 ng PAT, 64.52 µg total protein)

Lane 4: Nontransgenic soybean seed extract (64.52 µg total protein)

Lane 5: Molecular weight standard

Lane 6: PAT purified preparation from SYHT0H2 extract (10 ng PAT)¹

Lane 7: Microbially produced PAT (10 ng PAT)¹

Lane 8: Molecular weight standard

¹ Due to limitations in printer resolution, the faint band visible at approximately 43 kDa may not be visible on the printed copy.

4.2 Specific Enzymatic Activity

The microbially produced PAT showed a mean specific activity of 20.84 Units/mg PAT and the plant-produced PAT in crude SYHT0H2 soybean seed extract showed a mean specific activity of 30.58 Units/mg PAT (Table 3). To simulate the experimental extraction conditions of the plant-produced PAT, the microbially produced PAT was added to the nontransgenic, near-isogenic soybean seed extract. The specific activity for this sample was found to be 22.13 Units/mg PAT (Table 3). Correcting for the effect of the plant extract places the detected specific activities of the microbially produced and plant-produced PAT into comparable ranges (22.13 and 30.58 Units/mg PAT, respectively), with specific activity differing by $\leq 30\%$ between the two proteins (27.6%), thus confirming equivalent functional activity of both proteins.

TABLE 3 Specific enzymatic activity of the microbially produced and plant-produced PAT

	Assay replicate	PAT specific activity (U/mg PAT) ^a	Mean PAT specific activity (U/mg PAT)	RSD (%)
Plant-produced PAT	1	31.33	30.58	2.1
	2	30.22		
	3	30.20		
Microbially produced PAT	1	20.77	20.84	1.4
	2	20.60		
	3	21.16		
Nontransgenic extract + microbially produced PAT	1	20.69	22.13	5.8
	2	23.19		
	3	22.52		
Nontransgenic extract	1	< LOD ^b	< LOD ^b	—
	2	< LOD ^b		
	3	< LOD ^b		

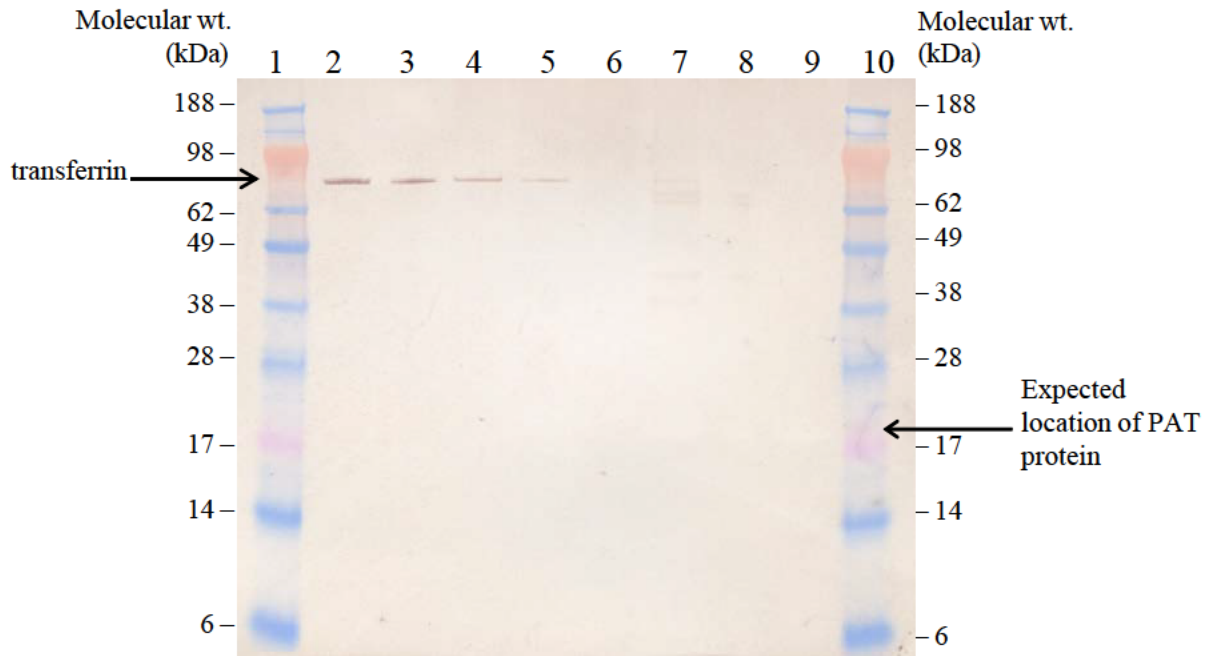
^a one unit of PAT activity is defined as the amount of enzyme required to acetylate 1 μ mol of phosphinothricin per minute (equivalent to 1 μ mol DTNB reduced or 1 μ mol TNB²⁻ produced per minute) under the described reaction conditions

^b LOD=15.3 μ M TNB²⁻ (Appendix A)

4.3 Glycosylation Analysis

Transferrin, the positive control, generated a visible band when applied to the gel at 100, 50, 25, and 10 ng (Figure 3, Lanes 2-5). Transferrin has a molecular weight of approximately 80 kDa and contains approximately 5% glycan moieties by weight. This corresponds to approximately 25 glucose equivalents per molecule (based on a calculated molecular weight of 162 Da for the glycan moiety). The band visualized for 10 ng of transferrin (Figure 3, Lane 5) represents 0.5 ng of glycan moieties. The maximum amount of PAT from both the microbial and plant sources loaded on the blot was 1000 ng. If 0.5 ng of glycan were detected in PAT, this would correspond to 0.05% by weight, or 0.064 glucose equivalents per molecule PAT (based on a calculated molecular weight of 20.6 kDa per molecule PAT). In other words, if PAT bands were detected as strongly as 10 ng of transferrin in Figure 3, Lane 5, this would indicate glycosylation of about one in 15 PAT molecules. No bands corresponding to the presence of glycosylated PAT were visible in the microbially produced (Figure 3, Lane 9) or plant-produced PAT samples (Figure 3, Lanes 7 and 8); therefore, the results support the conclusion that neither the microbially produced nor the plant-produced PAT is glycosylated.

FIGURE 3 Glycosylation analysis of a PAT purified preparation from SYHT0H2 soybean seed extract, and the microbially produced PAT



- Lane 1: Molecular weight standard
- Lane 2: Transferrin (positive control), 100 ng
- Lane 3: Transferrin (positive control), 50 ng
- Lane 4: Transferrin (positive control), 25 ng
- Lane 5: Transferrin (positive control), 10 ng
- Lane 6: Soybean trypsin inhibitor (negative control), 1000 ng
- Lane 7: PAT purified preparation from SYHT0H2 soybean seed extract, 1000 ng
- Lane 8: PAT purified preparation from SYHT0H2 soybean seed extract, 500 ng
- Lane 9: PAT in the microbially produced test substance, 1000 ng
- Lane 10: Molecular weight standard

4.4 Peptide Mass Mapping

The peptide mass mapping analysis of the microbially produced PAT was assessed in a separate study (Moens 2012) and yielded coverage of 76.5% of the total predicted PAT amino acid sequence (Figure 4). The analysis of a PAT purified preparation from SYHT0H2 soybean seed extract yielded coverage of 63.4% of the total predicted PAT amino acid sequence (Figure 5). The identified peptides corresponded to regions throughout the sequence of PAT. The results of the peptide mass mapping analysis confirmed the identification of the purified proteins from both sources as PAT. Apart from the cleavage of the N-terminal methionine of the plant-produced PAT, the peptide mass mapping results confirmed the intact N- and C-termini of the microbially produced and plant-produced PAT proteins, confirming the intactness of the proteins. The removal of the N-terminal methionine is a common process for many proteins that occurs during translation (Buchanan 2000, Walling 2006).

FIGURE 4 Amino acid sequence identified by peptide mass mapping analysis of the microbially produced PAT (Moens 2012)

```
1    MSPERRPVEI RPATAADMAA VCDIVNHYIE TSTVNFRTEP QTPQEWIDDL
51   ERLQDRYPWL VAEVEGVVAG IAYAGPWKAR NAYDWTVEST VYVSHRHQRL
101  GLGSTLYTHL LKSMEAQGFK SVVAVIGLPN DPSVRLHEAL GYTARGTLRA
151  AGYKHGGWHD VGFWQDFEL PAPPRPVRPV TQI
```

Identified PAT fragments are bold and underlined

FIGURE 5 Amino acid sequence identified by peptide mass mapping analysis of a PAT purified preparation from SYHT0H2 soybean seed extract

```
1    MSPERRPVEI RPATAADMAA VCDIVNHYIE TSTVNFRTEP QTPQEWIDDL
51   ERLQDRYPWL VAEVEGVVAG IAYAGPWKAR NAYDWTVEST VYVSHRHQRL
101  GLGSTLYTHL LKSMEAQGFK SVVAVIGLPN DPSVRLHEAL GYTARGTLRA
151  AGYKHGGWHD VGFWQDFEL PAPPRPVRPV TQI
```

Identified PAT fragments are bold and underlined

4.5 N-Terminal Amino Acid Sequence Analysis

The N-terminal amino acid sequence of the microbially produced PAT was determined in a separate study (Moens 2012), and found to be consistent with the predicted sequence. The N-terminal amino acid sequencing results of a PAT purified preparation from SYHT0H2 soybean seed extract were also consistent with the predicted sequence, confirming the identity of both proteins.

Predicted sequence:	MSPERRPVEIR
Microbially produced PAT (Moens 2012):	MSPER
Plant-produced PAT:	SPERRPVEIR

The N-terminal sequencing analysis revealed that the plant-produced PAT lacked the N-terminal methionine. The excision of the N-terminal methionine for the plant derived sequence is further confirmed by peptide mass mapping results (Figure 5).

4.6 Data Quality and Integrity

No circumstances occurred during the conduct of this study that would have adversely affected the quality or integrity of the data generated.

5.0 CONCLUSIONS

Western blot analysis of microbially produced and plant-produced PAT revealed immunoreactive bands that were consistent with the predicted molecular weight of the protein. Microbially produced PAT, in the presence of nontransgenic, near-isogenic soybean seed extract, and plant-produced PAT had comparable specific enzymatic activity. There was no evidence of post-translational glycosylation of microbially produced or plant-produced PAT. Peptide mass mapping analysis confirmed the identity and intactness of the proteins from both sources as PAT. Apart from the cleavage of the N-terminal methionine from the plant-produced protein, the N-terminal peptide of PAT from both sources was consistent with the predicted sequence.

The results of this study support the conclusion that microbially produced PAT is biochemically and functionally equivalent to plant-produced PAT, and provide the experimental evidence that the microbially produced PAT is a suitable surrogate for PAT produced in SYHT0H2 soybean.

6.0 REFERENCES

- Aitken A, Learmonth M. 1996. Protein Determination by UV Absorption. In *The Protein Protocols Handbook*. Walker JM, ed. Totowa, NJ: Humana Press. pp. 3-6.
- Buchanan BB, Gruissem W, Jones RL. 2000. Biochemistry and Molecular Biology of Plants. USA: Courier Companies, Inc. pp 434-453.
- Burgin K. 2011. *Event SYHT0H2 Soybean: Test and Control Substance Characterization of T₄, T₅, and T₆ Generations and Jack Soybean*. Report No. TK0055856 (unpublished). Research Triangle Park, NC: Syngenta Crop Protection, LLC.
- D'Halluin K, De Block M, Denecke J, Janssens J, Leemans J, Reynaerts A, Botterman J. 1992. The *bar* gene as selectable and screenable marker in plant engineering. *Methods Enzymol.* 216:415-426.
- Dreesen R. 2011. Stability of a protein during storage: PAT/pat batch 1995. Report No. BIOX-002_ProtQual_580 (unpublished). Ghent, Belgium: Bayer CropScience.
- Habeeb AFSA. 1972. Reaction of protein sulfhydryl groups with Ellman's reagent. *Methods Enzymol.* 25:457-464.
- Hill HD, Straka JG. 1988. Protein determination using bicinchoninic acid in the presence of sulfhydryl reagents. *Anal. Biochem* 170:203–208.
- Ingham DJ, Beer S, Money S, Hansen G. 2001. Quantitative real-time PCR assay for determining transgene copy number in transformed plants. *BioTechniques* 31:132–140.
- Moens S. 2012. Characterization of the PAT/pat protein batch n° 1995, produced in *Escherichia coli*. Report No. BBS11-010 (unpublished). Ghent, Belgium: Bayer CropScience.
- NIST. 2011. *The NIST Reference on Constants, Units, and Uncertainty*. Gaithersburg, MD: National Institute of Standards and Technology. <http://physics.nist.gov/cuu> (updated June 2, 2011).
- Riddles PW, Blakeley RL, Zerner B. 1979. Ellman's reagent: 5,5'-dithiobis(2-nitrobenzoic acid)—a reexamination. *Anal. Biochem.* 94:75-81.
- Riddles PW, Blakeley RL, Zerner B. 1983. Reassessment of Ellman's reagent. *Methods Enzymol.* 91:49-60.
- Song S. 2011. Single Laboratory Method Validation of Phosphinothricin Acetyltransferase (PAT) Enzymatic Activity Assay. Report No. TK0030218 (unpublished). Research Triangle Park, NC: Syngenta Crop Protection, LLC.

- Tijssen, P. 1985. Processing of data and reporting of results of enzyme immunoassays. In, *Practice and theory of enzyme immunoassays*. (Laboratory techniques in biochemistry and molecular biology, V. 15) Elsevier Science Publishers, Amsterdam, The Netherlands, pp. 385-421.
- Thompson CJ, Movva NR, Tichard R, Crameri R, Davies JE, Lauwereys M, Botterman J. 1987. Characterization of the herbicide-resistance gene bar from *Streptomyces hygroscopicus*. *EMBO J.* 6:2519–2523.
- US EPA. 1989. Good Laboratory Practices Standards. 40 CFR Part 160.
- Walling LL. 2006. Recycling or regulation? The role of amino-terminal modifying enzymes. *Current Opinion in Plant Biology* 9:227-233.

APPENDICES SECTION

APPENDIX A Enzymatic Activity Assay LOD Determination

Materials and Methods

The limit of detection (LOD) of PAT enzymatic activity for plant-matrix-based samples was determined by extracting four replicates of nontransgenic, near-isogenic soybean seed powder employing the same method as described in section §3.3.1 and examining the change in absorbance over time at 412 nm. Forty independently prepared replicates of reaction mixture (10 replicates per plant extract) were prepared. Each replicate had a total volume of 100 µl and contained 1 mM phosphinothricin, 1 mM acetyl CoA and 1 mM DTNB in 50 mM Tris, 2 mM EDTA, 0.5 mg/ml BSA, pH 7.5. The volume of nontransgenic soybean seed extract added to the reaction mixture for LOD determination was equivalent to the average volume of SYHT0H2 soybean seed extract added to the reaction mixture for PAT specific enzymatic activity measurement.

Prior to the addition of the nontransgenic extract, the reaction mixture was preincubated for 2 minutes at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$. After the addition of the nontransgenic extract to the reaction mixture, the assay was monitored spectrophotometrically by Molecular Devices SoftMax Pro® GxP software, version 5.4.1 at 412 nm at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ over 5 minutes with readings taken every 12 seconds, and the pathlength of the sample in each well was determined. For each sample, the difference in absorbance measurement between the time zero and 5-minute time point ($T_5 - T_0$) was calculated and corrected for the pathlength of each reaction well. To determine the LOD, the differences in absorbance of all 40 reaction blanks containing the nontransgenic extracts were averaged and the standard deviation was calculated. The LOD is equivalent to the average difference plus two times the standard deviation.

Results

The average absorbance difference of the reaction blanks was 0.1825 and the standard deviation was 0.0172 (Table A1). The average difference plus two times the standard deviation (0.2169) of the reaction blanks was then converted to a concentration of TNB^{2-} via the molar extinction constant ($14,150 \text{ M}^{-1} \text{ cm}^{-1}$) according to the Beer-Lambert law as indicated in §3.7.2. The LOD was found to be 216.9 milli OD, which is equivalent to $15.3 \mu\text{M}$.

To determine if a sample is below the LOD, the absorbance reading at time zero was subtracted from the absorbance reading at 5 minutes for each sample. If the difference was less than 216.9 milli OD then the sample was noted as being below the LOD.

TABLE A1 Results of the LOD determination

Assay replicate	(T ₅ -T ₀)/pathlength	Assay replicate	(T ₅ -T ₀)/pathlength
1	0.1646	21	0.2080
2	0.1784	22	0.1811
3	0.1735	23	0.1829
4	0.1672	24	0.1853
5	0.1715	25	0.1838
6	0.1689	26	0.1801
7	0.1752	27	0.1819
8	0.1750	28	0.1816
9	0.1784	29	0.1788
10	0.1786	30	0.1801
11	0.1763	31	0.2162
12	0.1679	32	0.2405 ^a
13	0.1675	33	0.2134
14	0.1665	34	0.2138
15	0.1653	35	0.2097
16	0.1645	36	0.2093
17	0.1623	37	0.2056
18	0.1617	38	0.2059
19	0.1633	39	0.2034
20	0.1636	40	0.2052

^a This data point is treated as an outlier as it falls above (Avg+ 3Stdev) and was not used to determine the LOD

Average OD ₄₁₂ (T ₅ -T ₀)	StDev (OD)	LOD	
		Avg + 2StDev (OD)	TNB ²⁻ (μM)
0.1825	0.0172	0.2169	15.3

APPENDIX B Peptide Mass Mapping and N-terminal Sequencing Phase Report

Phase Report Addendum 1 for Study TK0059680 Page 1 of 4



Peptide Mass Mapping and Edman Sequencing Phase Report

Study Number: TK0059680

Study Title: Comparison of Phosphinothricin Acetyltransferase (PAT) Protein Produced in Recombinant Escherichia coli and PAT Protein Produced in Event SYHT0H2 Derived Soybean Plants

Analytical Phase Study Plan no. 12011902

Analytical Phase Report no. 1203/23099 Addendum 1

Test Site: SGS M-SCAN LIMITED
2-3, Millars Business Centre, Fishponds Close, Wokingham, Berkshire, RG41 2TZ, UK

Principal Investigator: [REDACTED]
SGS M-SCAN LIMITED
2-3, Millars Business Centre, Fishponds Close, Wokingham, Berkshire, RG41 2TZ, UK
[REDACTED]

Study Director: [REDACTED]
3054 East Cornwallis Road
Research Triangle Park, NC 27709
[REDACTED]

Study Sponsor: Syngenta Crop Protection LLC
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419-8300 USA

Report prepared by [REDACTED] Date 28/6/12

Reviewed by [REDACTED] Date 28/6/12

Phase Report Addendum 1 for Study TK0059680 Page 2 of 4

Study Title: Comparison of Phosphinothricin Acetyltransferase (PAT) Protein
Produced in Recombinant Escherichia coli and PAT Protein Produced in
Event SYHT0H2 Derived Soybean Plants

Study Number: TK0059680

**Analytical Phase
Study Plan no** 12011902

**Analytical Phase
Report no** 1203/23099 Addendum 1

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

PEPTIDE MASS MAPPING AND EDMAN SEQUENCING PHASE

The work described in this analytical phase study report was conducted in accordance with the principles laid down in the Good Laboratory Practice Regulations, SI 3106 (1999) and subsequent Amendment SI 994 (2004) which are themselves based on the principles of good laboratory practice contained in Annex 2 to the Decision of the Council of the Organisation for Economic Co-Operation and Development (OECD), ENV/MC/CHEM (98)17, and the guidelines set forth "The Application of the OECD Principles of GLP to the Organisation and Management of Multi Site Studies", (ENV/JM/MONO(2002)9), OECD Monograph No.13.). They are in conformity with, and implement the requirements of, Directives 2004/10/EC and 2004/9/EC.

Signed:



(Principal Investigator)

Date:

28.6.12

Phase Report Addendum 1 for Study TK0059680 Page 3 of 4

QUALITY ASSURANCE STATEMENT**Report Number:** 1203/23099 Addendum one**Analytical Phase Study Plan Number:** 12011902**Protocol Number:** TK0059680

Title: Comparison of Phosphinothricin Acetyltransferase (PAT) Protein
Produced in Recombinant *Escherichia coli* and PAT Protein
Produced in Event SYHT0H2 Derived Soybean Plants.

Quality Assurance reviewed the study in accordance with the Good Laboratory Practice Regulations, 1999 SI 3106 and subsequent amendment (2004) SI No. 994, as administered by the UK Medicine and Healthcare Products Regulatory Agency.


The following phases of the study were inspected and the findings reported to study management:

<u>Date Inspected</u>	<u>Phase</u>	<u>Date written report made to Management and Principal Investigator</u>	<u>Date written report made to Study Director</u>
28 Jun 2012	Draft Analytical Phase Study Report Add One	28 Jun 2012	29 Jun 2012

Details of the analytical phase inspected are listed above. Additional laboratory procedures are inspected on a routine basis in accordance with SGS M-Scan Limited standard operating procedures.

Facility inspections are conducted according to an approved schedule as described in SGS M-Scan Ltd standard operating procedures.

As far as can be reasonably established, the report was considered to be an accurate reflection of the raw data generated during the conduct of the study.

Signed:

(Assistant QA Manager)
Date:

29 June 2012

1. **Reason for Addendum 1**

Following finalization of the Report the Sponsor requested that the address of the Study Sponsor was changed from

Test Facility/Sponsor SYNGENTA CROP Protection LLC
3054 East Cornwallis Road
Research Triangle Park, NC 27705

to

Study Sponsor Syngenta Crop Protection LLC
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419-8300 USA

In addition the GLP Compliance Statement was altered to include a space for it to be signed by the Principal Investigator and inclusion of Analytical Phase Study Plan No. and Report number.

This Report 1203/23099 Addendum 1 was produced to incorporate the amended Sponsor Address and allow for signature of the GLP Compliance Statement by the Principal Investigator and inclusion of Analytical Phase Study Plan No. and Report number.

There is no impact on the results generated or the GLP compliance of the study.



Peptide Mass Mapping and Edman Sequencing Phase Report

Study Number: TK0059680

Study Title: Comparison of Phosphinothricin Acetyltransferase (PAT) Protein Produced in Recombinant Escherichia coli and PAT Protein Produced in Event SYHT0H2 Derived Soybean Plants

Analytical Phase Study Plan no. 12011902

Analytical Phase Report no. 1203/23099final

Test Site: SGS M-SCAN LIMITED
2-3, Millars Business Centre, Fishponds Close, Wokingham, Berkshire, RG41 2TZ, UK

Principal Investigator: [REDACTED]
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Study Director: [REDACTED]
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[REDACTED]

Test Facility/Sponsor: SYNGENTA CROP Protection LLC
3054 East Cornwallis Road
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Report prepared by [REDACTED] Date 3/14/2012

Reviewed by [REDACTED] Date 3.5.12

Distribution List

Signed Original	GLP Archives (Syngenta Limited, Jealott's Hill)
2	[REDACTED] (Syngenta Limited, Jealott's Hill)
3	[REDACTED] (Study Director, Syngenta Crop Protection LLC)
4	GLP Archives (SGS M-Scan)

This phase report contains 86 pages

Study Title: Comparison of Phosphinothricin Acetyltransferase (PAT) Protein
Produced in Recombinant Escherichia coli and PAT Protein Produced in
Event SYHT0H2 Derived Soybean Plants

Study Number: TK0059680

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

PEPTIDE MASS MAPPING AND EDMAN SEQUENCING PHASE

The work described in this analytical phase study report was conducted in accordance with the principles laid down in the Good Laboratory Practice Regulations, SI 3106 (1999) and subsequent Amendment SI 994 (2004) which are themselves based on the principles of good laboratory practice contained in Annex 2 to the Decision of the Council of the Organisation for Economic Co-Operation and Development (OECD), ENV/MC/CHEM (98)17, and the guidelines set forth "The Application of the OECD Principles of GLP to the Organisation and Management of Multi Site Studies", (ENV/JM/MONO(2002)9), OECD Monograph No.13.). They are in conformity with, and implement the requirements of, Directives 2004/10/EC and 2004/9/EC.

Quality Assurance Statement

Phase Report for Study TK0059680 Page 4 of 86

QUALITY ASSURANCE STATEMENT**Study Number:** TK0059680**Report Number:** 1203/23099**Analytical Phase Study Number:** 12011902**Analytical Phase Title:** N-Terminal(Edman) sequencing and In-gel tryptic Digestion with peptide mass mapping using LC-ES-MS/MS analysis of phosphinothricin Acetyltransferase (PAT) protein produced in Event SYHT0H2 derived soybean plants.

Quality Assurance reviewed the study in accordance with the Good Laboratory Practice Regulations, 1999 SI 3106 and subsequent amendment (2004) SI No. 994, as administered by the UK Medicine and Healthcare Products Regulatory Agency.

The following phases of the study were inspected and the findings reported to study management:

<u>Date Inspected</u>	<u>Phase</u>	<u>Date written report made to Site Management and Principal Investigator</u>	<u>Date written report made to Study Director</u>
14 th Feb 2012	Draft Protocol	14 th Feb 2012	14 th Feb 2012
24 th Feb 2012	Sample receipt	24 th Feb 2012	3 rd May 2012
1 st Mar 2012	Sample receipt	2 nd Mar 2012	3 rd May 2012
14-15 th Mar 2012	In-gel Digest	10 th Apr 2012	10 th Apr 2012
19 th -22 nd Mar 2012	LCESMS/MS (Data Processing)	26 th Mar 2012	3 rd May 2012
1 st May 2012	Draft Report	3 rd May 2012	3 rd May 2012
3 rd May 2012	Final Report	3 rd May 2012	3 rd May 2012

Details of the analytical phase inspected are listed above. Additional laboratory procedures are inspected on a routine basis in accordance with SGS M-Scan Limited standard operating procedures.

Facility inspections are conducted according to an approved schedule as described in SGS M-Scan Ltd standard operating procedures.

As far as can be reasonably established, the report was considered to be an accurate reflection of the raw data generated during the conduct of the study.

Signed: 
(Quality Assurance Unit)

Date: 03 May 2012

Contents

Distribution List.....	2
GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT	3
Quality Assurance Statement	4
Contents	5
Summary	6
1. Purpose of Phase.....	7
2. Materials and Methods	7
2.1 Peptide Mass Mapping	7
2.1.1 Materials	7
2.1.2 SDS-PAGE analysis and enzymatic digestion	7
2.1.3 Liquid Chromatography-Mass Spectrometry / Mass Spectrometry (LC-MS/MS).....	9
2.1.4 Data Processing and Interpretation.....	9
2.2 Edman Sequencing	9
2.2.1 Materials	9
2.2.2 Pulsed-Liquid N-terminal Sequencing	10
2.2.3 Sample Analysis	10
2.2.4 Data Processing and Interpretation.....	10
3. Results and Discussion	11
3.1 Peptide Mass Mapping	11
3.2 Edman sequencing	12
3.2.1 N-Terminal Sequencing of β -Lactoglobulin Standard.....	12
3.2.2 N-Terminal Sequencing of phosphinothricin acetyltransferase (PAT) protein	13
4. Conclusions.....	13
5. Raw Data and Report Retention.....	13
6. Personnel	14
7. Study Phase Dates.....	14
8. Sample Disposal	14
9. References	14
10. Appendix	15
10.1 Peptide Mass Mapping Data for phosphinothricin acetyltransferase (PAT) protein from Event SYHT0H2	15
10.1.1 Mascot search parameters for the phosphinothricin acetyltransferase (PAT) protein from Event SYHT0H2.....	15
10.1.2 Manual Verification of Mascot Data.....	15
10.1.3 Example of a manually verified MS/MS spectrum of phosphinothricin acetyltransferase (PAT) protein from Event SYHT0H2.....	15
10.2 Complete Mascot search results.....	18
10.3 Edman Sequencing Data for β -lactoglobulin standard	51
10.4 Edman Sequencing Data for phosphinothricin acetyltransferase (PAT) protein from Event SYHT0H2	72

Summary

Samples of the phosphinothricin acetyltransferase (PAT) protein produced in Event SYHT0H2 derived soybean plants were received for peptide mass mapping analysis and N-terminal sequence analysis from Product Safety, Syngenta Crop Protection, LLC, Research Triangle Park, NC, USA. Prior to arrival, the proteins were separated by SDS-PAGE.

The phosphinothricin acetyltransferase (PAT) protein bands were excised from the gel, reduced, alkylated with iodoacetamide, and enzymatically digested using trypsin, chymotrypsin and Asp-N. A separate digestion was conducted with each enzyme. The digested samples were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) with a quadrupole time-of-flight mass spectrometer (Q-TOF Xevo, Waters) coupled to a nano LC chromatography instrument (Ultimate 3000 nano-HPLC, Dionex). The detected peptide spectra were searched using MASCOT® Software against a protein database containing the expected amino acid sequence of phosphinothricin acetyltransferase (PAT) protein. Peptide mass mapping analysis was conducted by SGS M-Scan Limited (Wokingham, UK).

The analysis of the plant produced phosphinothricin acetyltransferase (PAT) protein yielded coverage of 63.4% of the total predicted phosphinothricin acetyltransferase (PAT) protein amino acid sequence. The identified peptides corresponded to regions throughout the sequence of phosphinothricin acetyltransferase (PAT) protein including the N- and C-terminus of the protein. The results of the peptide mass mapping analysis confirmed the identification of the purified protein produced in Event SYHT0H2 derived soybean plants as phosphinothricin acetyltransferase (PAT) protein.

Additionally the N-terminal sequence of the phosphinothricin acetyltransferase (PAT) protein in Event SYHT0H2 derived soybean plants was determined and was found to be consistent with the predicted N-terminal sequence of the protein (though missing the methionine at the N-terminus), indicating positive identity of the phosphinothricin acetyltransferase (PAT) protein.

1. **Purpose of Phase**

To confirm the identity of the phosphinothricin acetyltransferase (PAT) protein produced in Event SYHT0H2 derived soybean plants using peptide mass mapping, and to determine the N-terminal sequence of this protein.

2. **Materials and Methods**

The study was performed according to the Analytical Phase Study Plan number 12011902.

2.1 **Peptide Mass Mapping**

2.1.1 **Materials**

One SDS-PAGE gel (EVENT SYHT0H2 PAT 20120221) containing PAT protein produced in Event SYHT0H2 derived soybean plants, was received from [REDACTED] (Syngenta Crop Protection LLC) on 24 Feb 2012 (Figure 2) and allocated a unique reference number as described below:

Product	SGS M-Scan No.
One SDS-PAGE gel (EVENT SYHT0H2 PAT 20120221) of phosphinothricin acetyltransferase (PAT) protein produced in Event SYHT0H2	99570

Samples were stored at 2-8 °C prior to analyses.

The expected amino acid sequence of phosphinothricin acetyltransferase (PAT) protein supplied by the study sponsor is shown in Figure 1 below.

```

MSPERRPVEIRPATAADMAAVCDIVNHYIE
TSTVNFRTPEQTPQEWIDDLERLQDRYPWL
VAEVEGVVAGIAYAGPWKARNAYDWTVEST
VYVSHRHQRLGLGSTLYTHLLKSMEAQGFK
SVVAVIGLPNDPSVRLHEALGYTARGTLRA
AGYKHGGWHDVGFQWQDFELPAPPRPVRPV
TQI

```

Figure 1. Amino acid sequence (M₁-I₁₈₃) of the protein phosphinothricin acetyltransferase (PAT)

2.1.2 **SDS-PAGE analysis and enzymatic digestion**

For peptide mass mapping analysis the main phosphinothricin acetyltransferase (PAT) protein bands from lanes 4, 5 and 6 (as indicated in Figure 2) were excised from the SDS-PAGE gel. The protein was reduced using dithiothreitol, alkylated with iodoacetamide and digested separately with trypsin, chymotrypsin and Asp-N. The digested samples, which were used for all subsequent analyses described in this report, were assigned the unique reference numbers shown in Table 1.

Sample name	Phosphinothricin acetyltransferase (PAT) protein from Event SYHT0H2		
Enzyme	<i>Trypsin</i>	Chymotrypsin	<i>Asp-N</i>
Gel band reference	4	5	6
Unique digest sample reference	99570Try	99570Chy	99570Asp

Table1. Shows the unique nomenclature for each of the samples submitted for mass spectrometric analysis

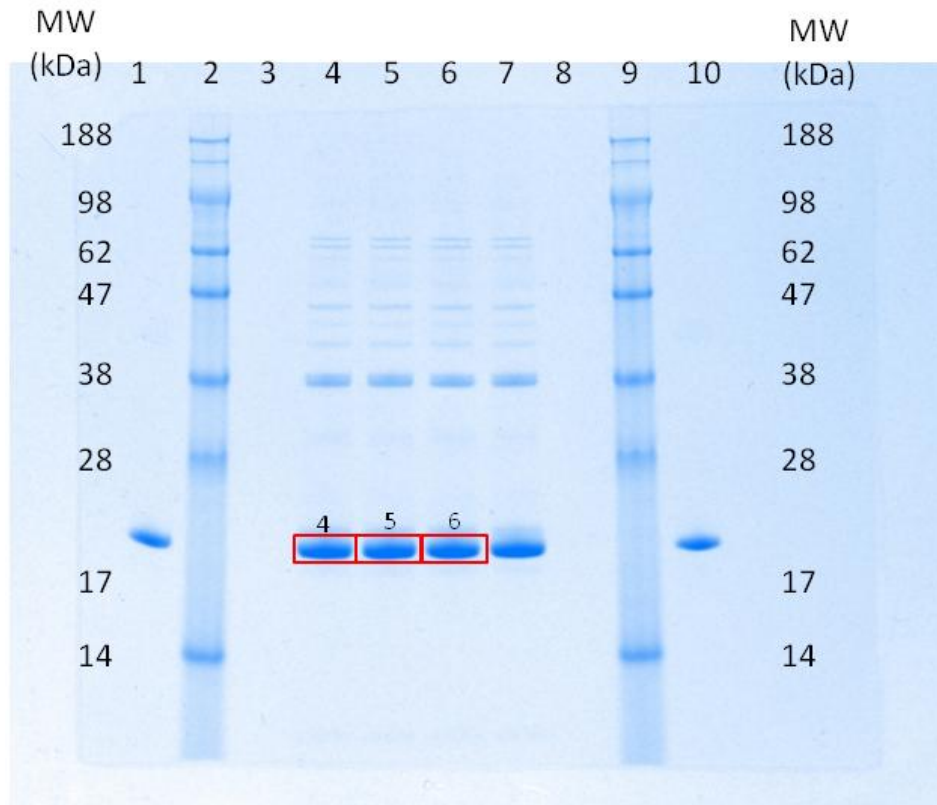


Figure 2. SDS-PAGE gel (EVENT SYHT0H2 PAT 20120221) of PAT protein produced in Event SYHT0H2 derived soybean plants. Lanes 2 & 9 MW markers. Lanes 4-7 phosphinothricin acetyltransferase (PAT) protein from Event SYHT0H2. Lanes 1 & 10 20kDa reference protein. The uniquely labelled bands were excised from the gel for enzymatic digestion.

2.1.3 Liquid Chromatography-Mass Spectrometry / Mass Spectrometry (LC-MS/MS)

All mass measurements were made using a quadrupole time-of-flight mass spectrometer (XEVO Q-TOFMS#YAA120). Samples and standards were delivered to the mass spectrometer via a nano-UPLC instrument (Ultimate 3000 nano-HPLC, Dionex). The instruments were controlled and data was collected using MassLynx software v4.1 (SCN 723). The mass spectrometer was operated in positive ion mode using a nanoflow ionisation source.

Samples were introduced into the mass spectrometer via an analytical capillary column (Waters C18 PepMap100 15cm, 3µm ID, 100Å). A flow rate of 350 nL min⁻¹ was used for all separations.

Prior to conducting the analysis the instrument was calibrated using the product ions from the fragmentation of Glu-Fibrinopeptide (100 pmol µL⁻¹, in 1:1 acetonitrile : 0.05% formic acid in UHQ water) introduced using the lockspray functionality of the Xevo Q-TOF at a flowrate of 3 µL min⁻¹. The spectra from the digest samples were lock-mass corrected using Glu-Fibrinopeptide. 100 pmol µL⁻¹. Glu-Fibrinopeptide was infused at a rate of 3 µL min⁻¹ and the lock mass spray channel was sampled every 60 seconds.

6 µL of each sample was loaded onto the nano-LC system. The gradient conditions for the separation are described in Table 2. The temperature of the autosampler tray was regulated at 8 °C and the column temperature was maintained at 30 °C throughout the course of these experiments.

Time (min)	Solvent B (%)
0.00	4
5	4
95	50
95.1	90
105	90
105.1	4
110	4

Table 2. Gradient conditions used for all sample injections. Solvent A was UHQ water containing 0.05 % by volume formic acid. Solvent B was 90% acetonitrile in UHQ water containing 0.05 % by volume formic acid modifier.

The mass spectrometer was scanned from m/z 50 to 3,000 using both high (MS^e) and low energy channels.

2.1.4 Data Processing and Interpretation

All MS/MS spectra were processed and lock mass corrected by Masslynx into RAW files, which were created using Masslynx version 4.1 from Waters. Using Mascot (version 2.2.06) peaklist files were searched against a database containing the amino acid sequence shown in Figure 1. The parameters used for Mascot searches are described in the Appendix (Section 10.1.1 and 10.1.4). The Mascot search results were manually verified using the acceptance criteria shown in the Appendix (Section 10.1.2)^{1,2}.

2.2 Edman Sequencing

2.2.1 Materials

Phosphinothricin acetyltransferase (PAT) protein bands, produced in Event SYHT0H2 derived soybean plants were received from the Sponsor on 1 March 2012 and given the unique SGS M-Scan number described (Table 3). The supplied phosphinothricin acetyltransferase (PAT) protein test samples were provided by the Study Sponsor blotted and stained on a PVDF membrane. The expected N-terminal sequence of phosphinothricin acetyltransferase (PAT) protein is shown in Figure 3 below.

Product	SGS M-Scan No.
Feb 27 2012 LKS SYHT0H2 purified PAT N-TERMINAL SEQUENCING	99740
Feb 27 2012 LKS SYHT0H2 purified PAT N-TERMINAL SEQUENCING	99741

Table 3. Shows the unique nomenclature for each of the samples submitted for Edman sequencing

MSPERRPVEIRPATAADMAAVCDIVNHYIE
TSTVNFRTPEQTPQEWIDDLERLQDRYPWL
VAEVEGVVAGIAYAGPWKARNAYDWTVEST
VYVSHRHQRLGLGSTLYTHLLKSMEAQGFK
SVVAVIGLPNDPSVRLHEALGYTARGTLRA
AGYKHGGWHDVGFWRQDFELPAPPRPV
TQI

Figure 3. Amino acid sequence (M₁-I₁₈₃) of phosphinothricin acetyltransferase (PAT) protein

Samples were stored at -20 °C (± 8 °C) prior to analyses.

2.2.2 Pulsed-Liquid N-terminal Sequencing

Edman degradation was performed on the supplied bands using an automated pulsed-liquid sequencer. Analyses were performed using the following equipment: Applied Biosystems Procise 492 automated N-terminal sequencer equipped with HPLC consisting of 140C pumps, a 200 Perkin Elmer series detector, a reverse phase column PTH-C18 cartridge 2.1 x 220 mm. Automated pulsed liquid sequencing for 10 residues of the N-terminal sequence was undertaken. Pulsed-liquid sequencing was carried out using standard PVDF pulsed liquid methodology.

2.2.3 Sample Analysis

Prior to phosphinothricin acetyltransferase (PAT) protein analysis, an aliquot (10pmol/10µl) of prepared β-lactoglobulin solution was loaded onto a glass fibre filter precycled with BioBrene Plus on to the Procise and 15 residues were sequenced by Edman degradation. The released phenylthiohydantoin (PTH-) amino acid derivatives were identified by reversed phase HPLC analysis.

Precut PVDF membrane blots containing phosphinothricin acetyltransferase (PAT) protein were loaded on to the Procise and 10 residues were sequenced by Edman degradation. The released phenylthiohydantoin (PTH-) amino-acid derivatives were identified by reversed-phase HPLC analysis.

2.2.4 Data Processing and Interpretation

All PTH-amino acids showing a typical sequencing pattern and above the standard limit of detection were assigned. The residues raw pmoles based on peak height were reported for information only (the technique is not strictly quantitative). Please note that the standard limit of detection for the sequencer is 2pmoles and therefore residues below 2pmoles were not reported.

3. Results and Discussion

3.1 Peptide Mass Mapping

The datafiles resulting from the nano-LC-MS/MS analyses of the Trypsin, chymotrypsin and Asp-N digests from the plant expressed phosphinothricin acetyltransferase (PAT) protein, as contained in Event SYHT0H2 derived soybean plants, were input into the Mascot Daemon interface software. Each individual digest datafile was searched against a database containing the amino acid sequence shown in Figure 1. Manual verification of 18 MS/MS spectra generated the sequence coverage map shown in Figure 4 for the phosphinothricin acetyltransferase (PAT) protein from Event SYHT0H2 derived soybean plants. Evidence for 63.4% of the amino acid sequence was obtained. (The complete list of peptides found by the Mascot searches are reported in the Appendix)

Phosphinothricin acetyltransferase (PAT) protein

```

1  M SPERRPVEIRPATAADMAAVCDIVNHYIE 30
31 TSTVNFRTEPQTPQEWIDDLERLQDRYPWL 60
61 VAEVEGVVAGIAYAGPWKARNAYDWTVEST 90
91 VYVSHRHQRLGLGSTLYTHLLKSMEAQGFK 120
121 SVVAVIGLPNDPSVRLHEALGYTARGTLRA 150
151 AGYKHGGWHDVGFWRQDFELPAPPRPVRPV 180
181 TQI 183
_

```

Figure 4. Amino acid sequence coverage map for the phosphinothricin acetyltransferase (PAT) protein purified from Event SYHT0H2 derived soybean plants. The sequence highlighted in red indicates unique peptides found in the Asp-N digest, green indicates Chymotrypsin and yellow indicates trypsin. Evidence for 63.4% of the amino acid sequence was obtained.

3.2 Edman sequencing

3.2.1 N-Terminal Sequencing of β -Lactoglobulin Standard

Pulsed-liquid sequencing was performed on a β -lactoglobulin standard. 15 residues were sequenced by Automatic Edman degradation. The data obtained from the analysis is shown in Section 10.3 and summarised in Table 4.

Residue Number	β -Lactoglobulin Standard	
	PTH-AA Sequence Observed	pmoles Observed*
1	L	5.4
2	I	5.1
3	V	4.6
4	T	3.6
5	Q	4.2
6	T	3.1
7	M	3.7
8	K	3.6
9	G	4.5
10	L	4.3
11	D	3.1
12	I	3.9
13	Q	3.6
14	K	3.1
15	V	3.4

Table 4. N-Terminal sequencing of β -lactoglobulin Standard

The data obtained for a standard of β -lactoglobulin were consistent with the following N-terminal sequence:

LIVTQ TMKGL DIQKV

The data obtained are consistent with the known sequence of β -lactoglobulin. The expected 19 PTH-amino acid peaks and the diphenylthiourea (dptu) peak were present in the chromatogram obtained from analysis of the standard mixture of PTH-amino acid. The run was completed successfully and all amino acids and dptu were identified correctly. The average background corrected repetitive yield was calculated for L, I and V as 95.9 %. This value is within the bounds of the acceptance criteria of >94%.

3.2.2 N-Terminal Sequencing of phosphinothricin acetyltransferase (PAT) protein

PVDF Pulsed-liquid sequencing was performed on plant expressed phosphinothricin acetyltransferase (PAT) protein, as contained in Event SYHT0H2 derived soybean plants (SGS M-Scan No. 99740 and 99741), as detailed in the phase plan. 10 residues were sequenced by Automatic Edman degradation. The data obtained from the analysis is shown in Section 10.4 and summarized in Table 5.

Residue Number	Phosphinothricin acetyltransferase (PAT) protein from Event SYHT0H2	
	PTH-AA Sequence Observed	pmoles Observed* **
1	S	19.7
2	P	13.6
3	E	15.3
4	R	12.2
5	R	18.3
6	P	10.1
7	V	10.5
8	E	10.5
9	I	9.0
10	R	8.4

* Calculation based on 10 pmoles containing standard mixture. For information only, as N-terminal sequencing is not a quantitative technique.

** Values below 2pmoles (based on 10 pmoles containing standard mixture) will not be reported. Please see the raw data for actual values.

Table 5. N-Terminal sequencing of Plant expressed phosphinothricin acetyltransferase (PAT) protein as contained in Event SYHT0H2 derived soybean plants

Edman sequencing data was obtained from the plant expressed phosphinothricin acetyltransferase (PAT) protein, as contained in Event SYHT0H2 derived soybean plants. The N-terminal sequence of the protein was confirmed to be *Ser-Pro-Glu-Arg-Arg-Pro-Val-Glu-Ile-Arg* consistent with the predicted N-terminal sequence of phosphinothricin acetyltransferase (PAT) protein, though missing the methionine at the N-terminus.

4. Conclusions

The combination of trypsin, chymotrypsin and Asp-N digestion with peptide mass mapping confirmed the identity of the phosphinothricin acetyltransferase (PAT) protein produced in Event SYHT0H2 derived soybean plants by producing evidence for the amino acid sequence of the protein. Evidence for 63.4% of the amino acid sequence was obtained for the phosphinothricin acetyltransferase (PAT) protein produced in Event SYHT0H2 derived soybean plants (Figure 4). The N-terminal amino acid sequence of phosphinothricin acetyltransferase (PAT) protein produced in Event SYHT0H2 derived soybean plants was confirmed to be the same as the predicted sequence (though missing the methionine at the N-terminus), indicating positive identity of phosphinothricin acetyltransferase (PAT) protein.

5. Raw Data and Report Retention

The working file and study report will be transferred to the Study Sponsor. The analytical phase study plan, a copy of the working file, and final analytical phase report will be stored in an archive as described by SOP GP-013, for inspection and reference purposes.

6. Personnel

Analytical work reported herein was conducted by:



7. Study Phase Dates

The experimental work was performed between 14th March 2012 and 31st March 2012. The interpretation was conducted between 19th March 2012 and 2nd April 2012.

8. Sample Disposal

It is anticipated that samples will be partially or completely destroyed during or as part of the analyses; any remaining sample will be disposed upon completion of the study (finalised report).

9. References

- 1) Bunkenborg J. and Matthiesen R. (2007) Interpretation of collision-induced fragmentation tandem mass spectra of post-translationally modified peptides. From *Methods in Molecular Biology*, vol 367: *Mass spectrometry data analysis in proteomics*. Edited by R. Matthiesen. pp.169-194
- 2) Chen Y., Kwon S.W., Kim S.C. and Zhao Y. (2005). Integrated Approach for Manual Evaluation of Peptides Identified by Searching Protein Sequence Databases with Tandem Mass Spectra. *Journal of Proteome Research* 4, 998-1005

10. **Appendix**

10.1 **Peptide Mass Mapping Data for phosphinothricin acetyltransferase (PAT) protein from Event SYHT0H2**

10.1.1 ***Mascot search parameters for the phosphinothricin acetyltransferase (PAT) protein from Event SYHT0H2***

The following Mascot parameters were used when searching the combined peaklist file for phosphinothricin acetyltransferase (PAT) protein purified from Event SYHT0H2.

Type of search	MS/MS Ion Search
Enzyme	Trypsin, Chymotrypsin or Asp-N, \pm Pro cleavage
Fixed Modifications	Carboxymethyl (C)
Variable modifications	Oxidation (M), Deamidation (NQ)
Mass Values	Monoisotopic
Protein Mass	Unrestricted
Peptide Mass Tolerance	\pm 0.8 Da
Fragment Mass Tolerance	\pm 0.8 Da
Missed Cleavages	up to 3

10.1.2 ***Manual Verification of Mascot Data***

In addition to the acceptance criteria and tolerances used in the interrogation of the databases by Mascot, manual verification of each peptide hit was also carried out. This includes checking the search results for unexpected cleavages, verifying the delta mass values versus calculated mass values and the inspection of the raw data to confirm the presence at significant levels of key b- and y"- fragmentation ions. Peptides identified by Mascot that do not meet these criteria are rejected.

10.1.3 ***Example of a manually verified MS/MS spectrum of phosphinothricin acetyltransferase (PAT) protein from Event SYHT0H2***

Figure A1 is a copy of the Mascot search results for the tryptic peptide TEPQTPQEWIDDLER and Figure A2 is a copy of the raw data from which this Mascot search result was derived. These figures represent an example of a single MS/MS spectrum and corresponding Mascot search result that was manually verified and accepted using the verification criteria described in Section 10.2. The MS/MS spectrum shows a clear evidence of a y- ion series the masses of which are highlighted in the table. The consecutive y- ions at m/z 417.2, 532.3, 647.3 and 760.4 were used to accept this spectrum.

Mascot Search Results: Peptide View

Page 1 of 1

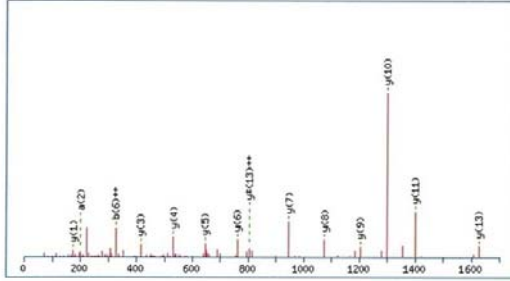
Mascot Search Results

Peptide View

MS/MS Fragmentation of **TEPQTPQEWIDDLER**
 Found in **00036 Syngenta**, Syngenta

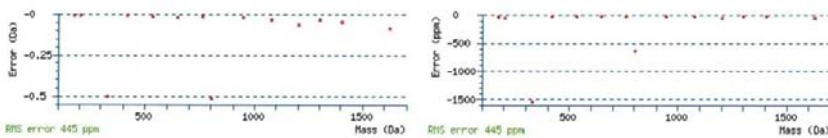
Match to Query 108: 1855.805068 from(928.909810,2+) intensity(4339.0000)
 Title: 89: Sum of 9 scans in range 3900 (rt=49.1336, f=2, i=1000) to 3908 (rt=49.2036, f=2, i=1008) [D:\Syngenta 23099\99570Tr.raw]
 Data file D:\Syngenta 23099\99570Tr.raw

Click mouse within plot area to zoom in by factor of two about that point
 Or, 0 1700



Monoisotopic mass of neutral peptide Mr(calc): 1855.8588
 Fixed modifications: Carbamidomethyl (C)
 Ions Score: 85 Expect: 1.3e-009
 Matches (Bold Red): 14/156 fragment ions using 17 most intense peaks

#	a	a ⁺⁺	a ⁺	a ⁺⁺⁺	b	b ⁺⁺	b ⁺	b ⁺⁺⁺	Seq.	y	y ⁺⁺	y ⁺	y ⁺⁺⁺	#
1	74.0600	37.5337			102.0550	51.5311			T					15
2	203.1026	102.0550			231.0975	116.0524			E	1755.8184	878.4128	1738.7919	869.8996	14
3	300.1554	150.5813			328.1503	164.5788			P	1626.7758	813.8916	1609.7493	805.3783	13
4	428.2140	214.6106	411.1874	206.0974	456.2089	228.6081	439.1823	220.0948	Q	1529.7231	765.3652	1512.6965	756.8519	12
5	529.2617	265.1345	512.2351	256.6212	557.2566	279.1319	540.2300	270.6186	T	1401.6645	701.3359	1384.6379	692.8226	11
6	626.3144	313.6608	609.2879	305.1476	654.3093	327.6583	637.2828	319.1450	P	1300.6168	650.8120	1283.5903	642.2988	10
7	754.3730	377.6901	737.3464	369.1769	782.3679	391.6876	765.3414	383.1743	Q	1203.5640	602.2857	1186.5375	593.7724	9
8	883.4156	442.2114	866.3890	433.6982	911.4105	456.2089	894.3840	447.6956	E	1075.5055	538.2564	1058.4789	529.7431	8
9	1069.4949	535.2511	1052.4684	526.7378	1097.4898	549.2485	1080.4633	540.7353	W	946.4629	473.7351	929.4363	465.2218	7
10	1182.5790	591.7931	1165.5524	583.2798	1210.5739	605.7906	1193.5473	597.2773	I	760.3836	380.6954	743.3570	372.1821	6
11	1297.6059	649.3066	1280.5794	640.7933	1325.6008	663.3041	1308.5743	654.7908	D	647.2995	324.1534	630.2729	315.6401	5
12	1412.6329	706.8201	1395.6063	698.3068	1440.6278	720.8175	1423.6012	712.3042	D	532.2726	266.6399	515.2460	258.1266	4
13	1525.7169	763.3621	1508.6904	754.8488	1553.7118	777.3596	1536.6853	768.8463	L	417.2456	209.1264	400.2191	200.6132	3
14	1654.7595	827.8834	1637.7330	819.3701	1682.7544	841.8809	1665.7279	833.3676	E	304.1615	152.5844	287.1350	144.0711	2
15									R	175.1190	88.0631	158.0924	79.5498	1



NCBI BLAST search of **TEPQTPQEWIDDLER**
 (Parameters: blastp, nr protein database, expect=20000, no filter, PAM30)
 Other BLAST [web gateways](#)

All matches to this query

Score	Mr(calc):	Delta	Sequence
85.0	1855.8588	-0.0537	TEPQTPQEWIDDLER

Mascot: <http://www.matrixscience.com/>

mhtml:file:///K:\Syngenta Mascot Exports\Tryp 1.mht

29/03/2012

Figure A1. Example of a manually verified MS/MS spectrum from phosphinothricin acetyltransferase (PAT) protein produced in Event SYHT0H2 for the tryptic peptide TEPQTPQEWIDDLER.

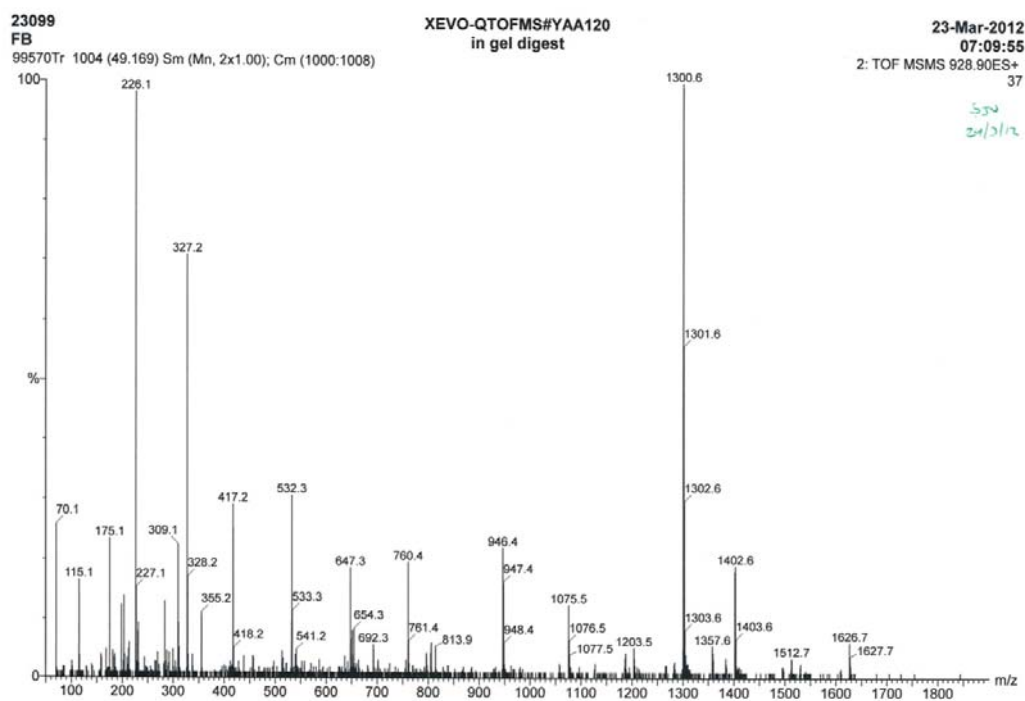


Figure A2. A copy of the raw data (without lock mass correction) from phosphinothricin acetyltransferase (PAT) protein produced in Event SYHT0H2 for the tryptic peptide *TEPQTPQEWIDDLER*. The Mascot search result shown in Figure A1 was derived from this data.

10.2 Complete Mascot search results

Mascot Search Results: Protein View

Page 1 of 1

Mascot Search Results

Protein View

Match to: **00036 Syngenta** Score: **189****Syngenta**

Found in search of D:\Syngenta 23099\99570Tr.raw

Nominal mass (M_r): **20662**; Calculated pI value: **6.37**NCBI BLAST search of **00036 Syngenta** against nrUnformatted [sequence string](#) for pasting into other applications

Fixed modifications: Carbamidomethyl (C)

Variable modifications: Deamidated (NQ), Oxidation (M)

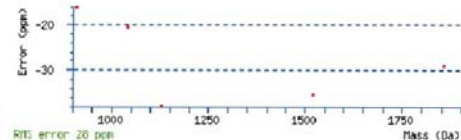
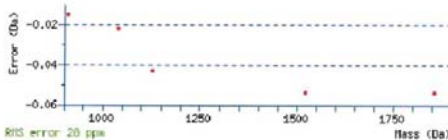
Cleavage by Trypsin/P: cuts C-term side of KR

Sequence Coverage: **31%**Matched peptides shown in **Bold Red**

1 MSPERRPVEI RPATAADMAA VCDIVNHYIE TSTVNER**TEP QTPQEWIDDL**
51 **ERLQDRYPWL** VAEVEGVVAG IAYAGPWKAR NAYDWTVEST VYVSHRHQL
101 GLGSTLYTHL LKSMQAQGFK **SVVAVIGLFPN DPSVRLREAL GYTARGTLRA**
151 AGYKHGGWHD VGFQWR**DFEL PAPPFPVRPV TQI**

Sort Peptides By ☒ Residue Number ☐ Increasing Mass ☐ Decreasing Mass

Start - End	Observed	Mr (expt)	Mr (calc)	Delta	Miss	Sequence
38 - 52	928.9098	1855.8051	1855.8588	-0.0537	0	R.TEPQTPQEWIDDLER.L (Ions score 85)
121 - 135	761.9062	1521.7978	1521.8515	-0.0537	0	K.SVVAVIGLFPNDPSVR.L (Ions score 65)
136 - 145	1130.5525	1129.5452	1129.5880	-0.0428	0	R.LREALGYTAR.G (Ions score 43)
167 - 175	1041.5150	1040.5077	1040.5291	-0.0214	0	R.DFELPAPPRA.P (Ions score 28)
176 - 183	909.5369	908.5297	908.5444	-0.0147	1	R.FVRPVTQI.- (Ions score 20)

Mascot: <http://www.matrixscience.com/>

Mascot Search Results: Peptide View

Page 1 of 1

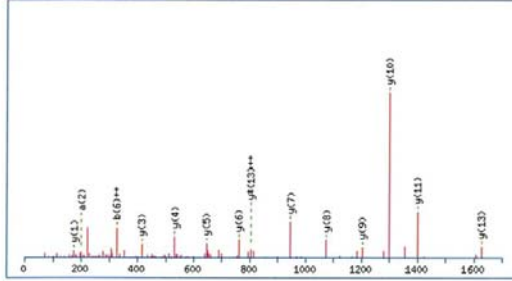
**(MATRIX)
(SCIENCE)** Mascot Search Results

Peptide View

MS/MS Fragmentation of **TEPQTPOEWIDDLER**
Found in **00036 Syngenta**, SyngentaMatch to Query 108: 1855.805068 from(928 909810,2+) intensity(4339.0000)
Title: 89: Sum of 9 scans in range 3900 (rt=49.1336, f=2, i=1000) to 3908 (rt=49.2036, f=2, i=1008) [D:\Syngenta 23099\99570Tr.raw]
Data file D:\Syngenta 23099\99570Tr.raw

Click mouse within plot area to zoom in by factor of two about that point

Or, Plot from 0 to 1700 Da Full range



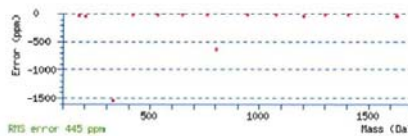
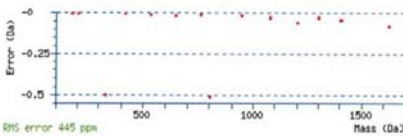
Monoisotopic mass of neutral peptide Mr(calc): 1855.8588

Fixed modifications: Carbamidomethyl (C)

Ions Score: 85 Expect: 1.3e-009

Matches (Bold Red): 14/156 fragment ions using 17 most intense peaks

#	a	a++	a*	a***	b	b++	b*	b***	Seq.	y	y++	y*	y***	#
1	74.0600	37.5337			102.0550	51.5311			T					15
2	203.1026	102.0550			231.0975	116.0524			E	1755.8184	878.4128	1738.7919	869.8996	14
3	300.1554	150.5813			328.1503	164.5788			P	1626.7758	813.8916	1609.7493	805.3783	13
4	428.2140	214.6106	411.1874	206.0974	456.2089	228.6081	439.1823	220.0948	Q	1529.7231	765.3652	1512.6965	756.8519	12
5	529.2617	265.1345	512.2351	256.6212	557.2566	279.1319	540.2300	270.6186	T	1401.6645	701.3359	1384.6379	692.8226	11
6	626.3144	313.6608	609.2879	305.1476	654.3093	327.6583	637.2828	319.1450	P	1300.6168	650.8120	1283.5903	642.2988	10
7	754.3730	377.6901	737.3464	369.1769	782.3679	391.6876	765.3414	383.1743	Q	1203.5640	602.2857	1186.5375	593.7724	9
8	883.4156	442.2114	866.3890	433.6982	911.4105	456.2089	894.3840	447.6956	E	1075.5055	538.2564	1058.4789	529.7431	8
9	1069.4949	535.2511	1052.4684	526.7378	1097.4898	549.2485	1080.4633	540.7353	W	946.4629	473.7351	929.4363	465.2218	7
10	1182.5790	591.7931	1165.5524	583.2798	1210.5739	605.7906	1193.5473	597.2773	I	760.3836	380.6954	743.3570	372.1821	6
11	1297.6059	649.3066	1280.5794	640.7933	1325.6008	663.3041	1308.5743	654.7908	D	647.2995	324.1534	630.2729	315.6401	5
12	1412.6329	706.8201	1395.6063	698.3068	1440.6278	720.8175	1423.6012	712.3042	D	532.2726	266.6399	515.2460	258.1266	4
13	1525.7169	763.3621	1508.6904	754.8488	1553.7118	777.3596	1536.6853	768.8463	L	417.2456	209.1264	400.2191	200.6132	3
14	1654.7595	827.8834	1637.7330	819.3701	1682.7544	841.8809	1665.7279	833.3676	E	304.1615	152.5844	287.1350	144.0711	2
15									R	175.1190	88.0631	158.0924	79.5498	1

NCBI BLAST search of **TEPQTPOEWIDDLER**
(Parameters: blastp, nr protein database, expect=20000, no filter, PAM30)
Other BLAST [web gateways](#)

All matches to this query

Score	Mr(calc)	Delta	Sequence
85.0	1855.8588	-0.0537	TEPQTPOEWIDDLER

Mascot: <http://www.matrixscience.com/>

mhtml:file:///K:\Syngenta Mascot Exports\Tryp 1.mht

29/03/2012

Mascot Search Results: Peptide View

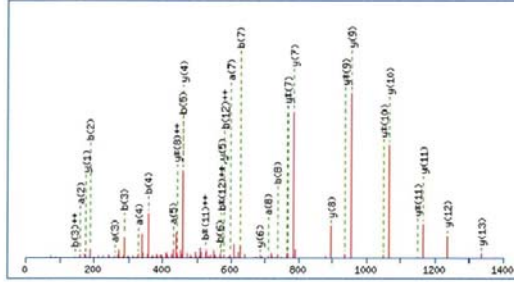
Page 1 of 1

**(MATRIX)
(SCIENCE)** Mascot Search Results

Peptide View

MS/MS Fragmentation of **SVVAVIGLPNDPSVR**
Found in **00036 Syngenta**, SyngentaMatch to Query 85: 1521.797768 from(761.906160,2+) intensity(88671.0000)
Title: 71: Sum of 9 scans in range 3114 (rt=38.9262, f=2, i=775) to 3154 (rt=39.2761, f=2, i=783) [D:\Syngenta 23099\99570Tr.raw]
Data file D:\Syngenta 23099\99570Tr.raw

Click mouse within plot area to zoom in by factor of two about that point

Or, Plot from 0 to 1400 Da 

Mascot Search Results: Peptide View

Page 1 of 2

Mascot Search Results

53
29/3/12

Peptide ViewMS/MS Fragmentation of **LHEALGYTAR**Found in **00036 Syngenta**, Syngenta

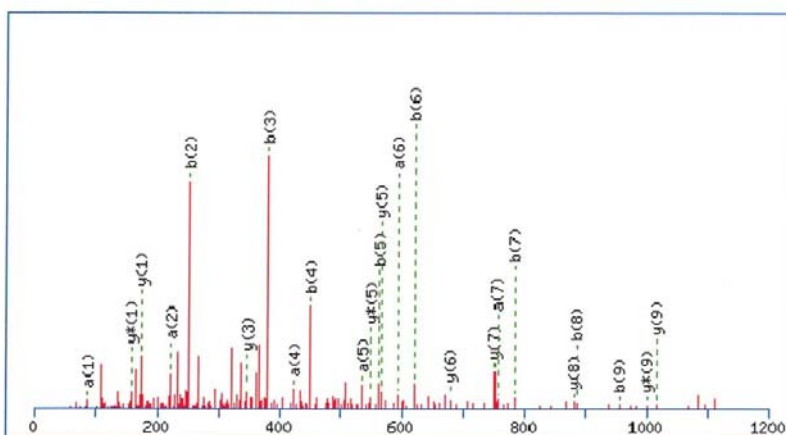
Match to Query 73: 1129.545224 from(1130.552500,1+) intensity(47721.0000)

Title: 9: Sum of 18 scans in range 1199 (rt=17.2498, f=2, i=178) to 1240 (rt=17.6086, f=3, i=72)

[D:\Syngenta 23099\99570Tr.raw]

Data file D:\Syngenta 23099\99570Tr.raw

Click mouse within plot area to zoom in by factor of two about that point

Or, 0 1200 

Monoisotopic mass of neutral peptide Mr(calc): 1129.5880

Fixed modifications: Carbamidomethyl (C)

Ions Score: 43 Expect: 2.3e-006

Matches (**Bold Red**): 24/36 fragment ions using 53 most intense peaks

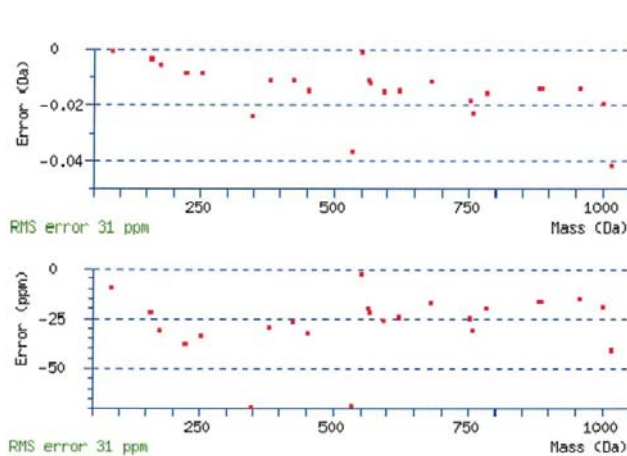
#	a	b	Seq.	y	y*	#
1	86.0964	114.0913	L			10
2	223.1553	251.1503	H	1017.5112	1000.4847	9
3	352.1979	380.1928	E	880.4523	863.4258	8
4	423.2350	451.2300	A	751.4097	734.3832	7
5	536.3191	564.3140	L	680.3726	663.3461	6
6	593.3406	621.3355	G	567.2885	550.2620	5
7	756.4039	784.3988	Y	510.2671	493.2405	4
8	857.4516	885.4465	T	347.2037	330.1772	3
9	928.4887	956.4836	A	246.1561	229.1295	2
10			R	175.1190	158.0924	1

mhtml:file://K:\Syngenta Mascot Exports\Tryp 3.mht

29/03/2012

Mascot Search Results: Peptide View

Page 2 of 2

NCBI BLAST search of [LHEALGYTAR](#)

(Parameters: blastp, nr protein database, expect=20000, no filter, PAM30)

Other BLAST [web gateways](#)

All matches to this query

Score	Mr(calc):	Delta	Sequence
43.3	1129.5880	-0.0428	LHEALGYTAR

Mascot: <http://www.matrixscience.com/>

mhtml:file:///K:/Syngenta Mascot Exports/Tryp 3.mht

29/03/2012

Mascot Search Results: Peptide View

Page 1 of 2

Mascot Search Results

573
27/5/12

Peptide ViewMS/MS Fragmentation of **DFELPAPPR**Found in **00036 Syngenta**, Syngenta

Match to Query 56: 1040.507724 from(1041.515000,1+) intensity(1810.0000)

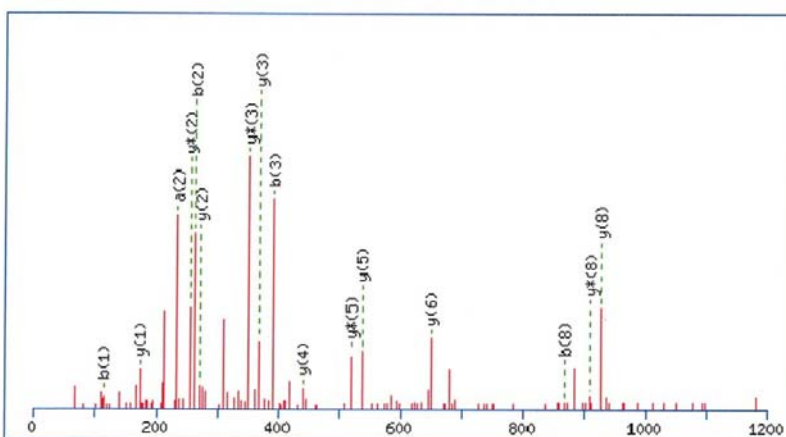
Title: 66: Sum of 8 scans in range 2881 (rt=35.9024, f=6, i=136) to 2916 (rt=36.2086, f=6, i=143)

[D:\Syngenta 23099\99570Tr.raw]

Data file D:\Syngenta 23099\99570Tr.raw

Click mouse within plot area to zoom in by factor of two about that point

Or, Plot from 0 to 1200 Da Full range



Monoisotopic mass of neutral peptide Mr(calc): 1040.5291

Fixed modifications: Carbamidomethyl (C)

Ions Score: 28 Expect: 0.00052

Matches (**Bold Red**): 16/32 fragment ions using 56 most intense peaks

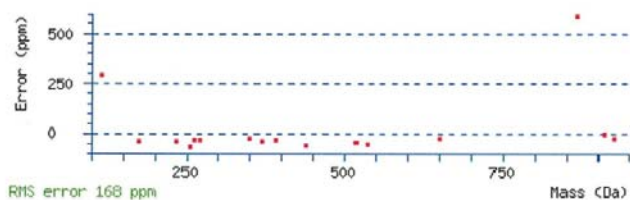
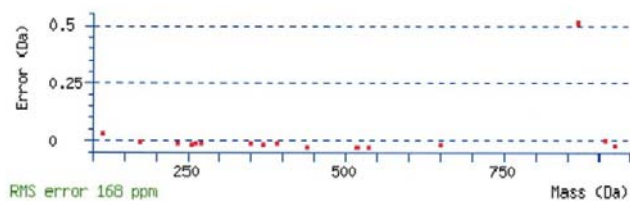
#	a	b	Seq.	y	y*	#
1	88.0393	116.0342	D			9
2	235.1077	263.1026	F	926.5094	909.4829	8
3	364.1503	392.1452	E	779.4410	762.4145	7
4	477.2344	505.2293	L	650.3984	633.3719	6
5	574.2871	602.2821	P	537.3144	520.2878	5
6	645.3243	673.3192	A	440.2616	423.2350	4
7	742.3770	770.3719	P	369.2245	352.1979	3
8	839.4298	867.4247	P	272.1717	255.1452	2
9			R	175.1190	158.0924	1

mhtml:file://K:\Syngenta Mascot Exports\Tryp 4.mht

29/03/2012

Mascot Search Results: Peptide View

Page 2 of 2

NCBI BLAST search of [DFELPAPPR](#)

(Parameters: blastp, nr protein database, expect=20000, no filter, PAM30)

Other BLAST [web gateways](#)

All matches to this query

Score	Mr(calc):	Delta	Sequence
28.3	1040.5291	-0.0214	DFELPAPPR

Mascot: <http://www.matrixscience.com/>

Mascot Search Results: Peptide View

Page 1 of 2

Mascot Search Results

532
29/3/12

Peptide ViewMS/MS Fragmentation of **PVRPVTQI**Found in **00036 Syngenta**, Syngenta

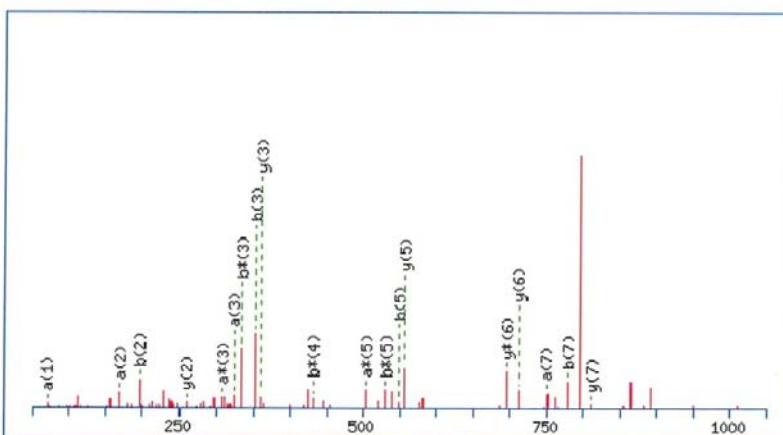
Match to Query 47: 908.529664 from(909.536940,1+) intensity(3035.0000)

Title: 21: Sum of 9 scans in range 1449 (rt=19.7032, f=5, i=68) to 1481 (rt=19.9832, f=5, i=76)

[D:\Syngenta 23099\99570Tr.raw]

Data file D:\Syngenta 23099\99570Tr.raw

Click mouse within plot area to zoom in by factor of two about that point

Or, 50 1050 

Monoisotopic mass of neutral peptide Mr(calc): 908.5444

Fixed modifications: Carbamidomethyl (C)

Ions Score: 20 Expect: 0.0011

Matches (**Bold Red**): 19/37 fragment ions using 50 most intense peaks

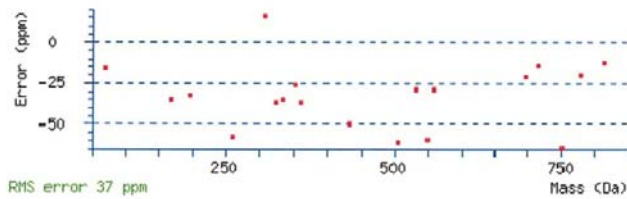
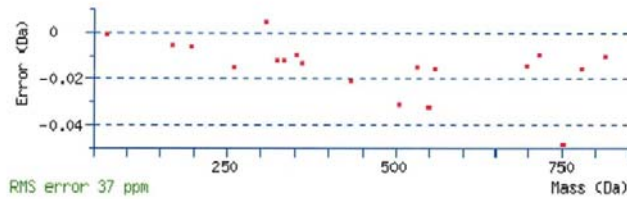
#	a	a*	b	b*	Seq.	y	y*	#
1	70.0651		98.0600		P			8
2	169.1335		197.1285		V	812.4989	795.4723	7
3	325.2346	308.2081	353.2296	336.2030	R	713.4305	696.4039	6
4	422.2874	405.2609	450.2823	433.2558	P	557.3293	540.3028	5
5	521.3558	504.3293	549.3507	532.3242	V	460.2766	443.2500	4
6	622.4035	605.3770	650.3984	633.3719	T	361.2082	344.1816	3
7	750.4621	733.4355	778.4570	761.4305	Q	260.1605	243.1339	2
8					I	132.1019		1

mhtml:file://K:\Syngenta Mascot Exports\Tryp 5.mht

29/03/2012

Mascot Search Results: Peptide View

Page 2 of 2

NCBI BLAST search of [PVRPVTQI](#)

(Parameters: blastp, nr protein database, expect=20000, no filter, PAM30)

Other BLAST [web gateways](#)

All matches to this query

Score	Mr(calc):	Delta	Sequence
19.6	908.5444	-0.0147	PVRPVTQI

Mascot: <http://www.matrixscience.com/>

mhtml:file://K:\Syngenta Mascot Exports\Tryp 5.mht

29/03/2012

Mascot Search Results: Protein View

Page 1 of 1

**(MATRIX)
SCIENCE** Mascot Search Results55-
27/3/12

Protein View

Match to: 00036 Syngenta Score: 180

Syngenta

Found in search of D:\Syngenta 23099\99570Asp.raw

Nominal mass (M_r): 20663; Calculated pI value: 6.37

NCBI BLAST search of 00036 Syngenta against nr

Unformatted [sequence string](#) for pasting into other applications

Fixed modifications: Carboxymethyl (C)

Variable modifications: Deamidated (NQ), Oxidation (M)

Cleavage by Asp-N_ambic: cuts N-term side of DE

Sequence Coverage: 36%

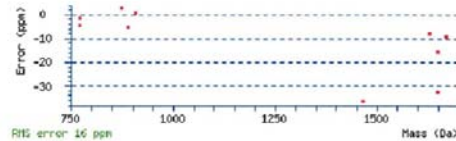
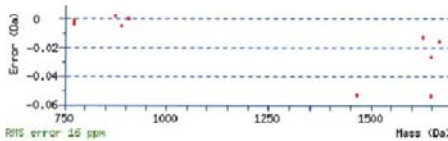
Matched peptides shown in **Bold Red**

1 **MSPERRPVEI** RPATAADMAA VCDIVNHYIE TSTVNFRTEP QTPQEWIDDL
51 **ERLQDRYPWL** VAEVEGVVAG IAYAGPWKAR NAYDWTVEST VYVSHRIQRL
101 GLGSTLYTHL LKSM**EAQGFK** SVVAVIGLPN DPSVRLHEAL GYTARGTLRA
151 AGYKHGGWHD **VGFWRQRFEL** PAPPFRVPV **TQI**

Show predicted peptides also

Sort Peptides By ☒ Residue Number ☐ Increasing Mass ☐ Decreasing Mass

Start	End	Observed	Mr (expt)	Mr (calc)	Delta	Miss	Sequence
2	16	550.6230	1648.8472	1648.9009	-0.0537	2	M.SPERRPVEIRPATAA.D (ions score 23)
2	16	825.4446	1648.8746	1648.9009	-0.0262	2	M.SPERRPVEIRPATAA.D (ions score 29)
4	16	489.2616	1464.7629	1464.8161	-0.0532	1	P.ERRPVEIRPATAA.D (ions score 20)
23	29	873.4490	872.4417	872.4392	0.0025	0	C.DIVNHYI.E (ions score 19)
48	54	888.4373	887.4300	887.4348	-0.0049	2	I.DDLERLQ.D (ions score 33)
49	54	773.4118	772.4046	772.4079	-0.0033	1	D.DLERLQ.D (ions score 28)
49	54	773.4141	772.4068	772.4079	-0.0011	1	D.DLERLQ.D (ions score 35)
115	130	614.9475	1627.8805	1627.8934	-0.0128	0	M.EAQGFKSVVAVIGLPN.D (ions score 53)
160	166	907.4426	906.4354	906.4348	0.0005	0	H.DVGWQR.D (ions score 36)
169	183	835.4832	1668.9518	1668.9675	-0.0157	0	F.ELPAPPFRVPVTQI.- (ions score 22)

Mascot: <http://www.matrixscience.com/>

Mascot Search Results: Peptide View

Page 1 of 1

Mascot Search Results

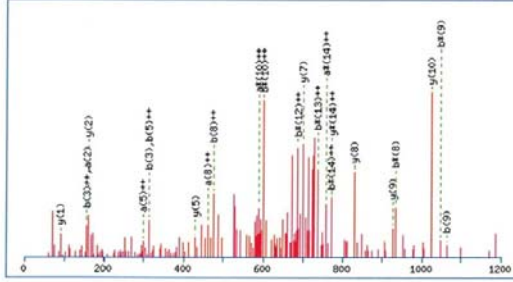
Peptide View

MS/MS Fragmentation of **SPERRPVEIRPATAA**
 Found in **00036 Syngenta**, Syngenta

Match to Query 100: 1648.847202 from(550.623010,3+) intensity(46558.0000)
 Title: 10: Sum of 9 scans in range 1104 (rt=15.6411, f=2, i=204) to 1112 (rt=15.7111, f=2, i=212) [D:\Syngenta 23099\99570\Asp.raw]
 Data file D:\Syngenta 23099\99570\Asp.raw

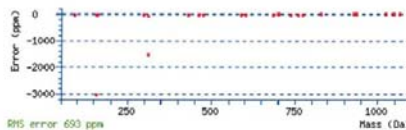
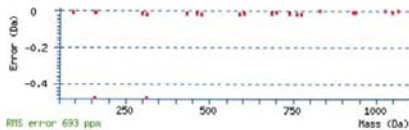
Click mouse within plot area to zoom in by factor of two about that point

Or, Plot from 0 to 1200 Da



Monoisotopic mass of neutral peptide Mr(calc): 1648.9009
 Fixed modifications: Carboxymethyl (C)
 Ions Score: 23 Expect: 0.00096
 Matches (Bold Red): 24/146 fragment ions using 37 most intense peaks

#	a	a ⁺⁺	a ⁺	a ⁺⁺⁺	b	b ⁺⁺	b ⁺	b ⁺⁺⁺	Seq.	y	y ⁺⁺	y ⁺	y ⁺⁺⁺	#
1	60.0444	30.5258			88.0393	44.5233			S					15
2	157.0972	79.0522			185.0921	93.0497			P	1562.8762	781.9417	1545.8496	773.4284	14
3	286.1397	143.5735			314.1347	157.5710			E	1465.8234	733.4153	1448.7968	724.9021	13
4	442.2409	221.6241	425.2143	213.1108	470.2358	235.6215	453.2092	227.1082	R	1336.7808	668.8940	1319.7542	660.3808	12
5	598.3420	299.6746	581.3154	291.1613	626.3369	313.6721	609.3103	305.1588	R	1180.6797	590.8435	1163.6531	582.3302	11
6	695.3947	348.2010	678.3682	339.6877	723.3896	362.1985	706.3631	353.6852	P	1024.5786	512.7929	1007.5520	504.2796	10
7	794.4631	397.7352	777.4366	389.2219	822.4581	411.7327	805.4315	403.2194	V	927.5258	464.2665	910.4993	455.7533	9
8	923.5057	462.2565	906.4792	453.7432	951.5007	476.2540	934.4741	467.7407	E	828.4574	414.7323	811.4308	406.2191	8
9	1036.5898	518.7985	1019.5633	510.2853	1064.5847	532.7960	1047.5582	524.2827	I	699.4148	350.2110	682.3883	341.6978	7
10	1192.6909	596.8491	1175.6644	588.3358	1220.6858	610.8466	1203.6593	602.3333	R	586.3307	293.6690	569.3042	285.1557	6
11	1289.7437	645.3755	1272.7171	636.8622	1317.7386	659.3729	1300.7120	650.8597	P	430.2296	215.6185			5
12	1360.7808	680.8940	1343.7542	672.3808	1388.7757	694.8915	1371.7492	686.3782	A	333.1769	167.0921			4
13	1461.8285	731.4179	1444.8019	722.9046	1489.8234	745.4153	1472.7968	736.9021	T	262.1397	131.5735			3
14	1532.8656	766.9364	1515.8390	758.4232	1560.8605	780.9339	1543.8340	772.4206	A	161.0921	81.0497			2
15									A	90.0550	45.5311			1



NCBI BLAST search of **SPERRPVEIRPATAA**
 (Parameters: blastp, nr protein database, expect=20000, no filter, PAM30)
 Other BLAST [web gateways](#)

All matches to this query

Score	Mr(calc)	Delta	Sequence
23.2	1648.9009	-0.0537	SPERRPVEIRPATAA

Mascot: <http://www.matrixscience.com/>

mhtml:file://K:\Syngenta Mascot Exports\AspN 1.mht

29/03/2012

Mascot Search Results: Peptide View

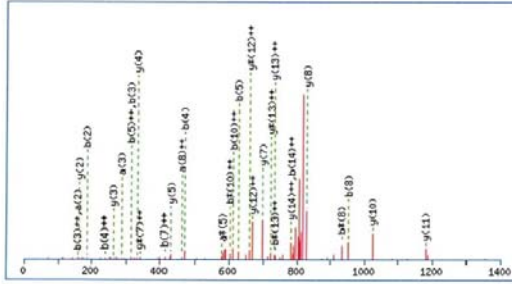
Page 1 of 1

**(MATRIX)
(SCIENCE)** Mascot Search Results

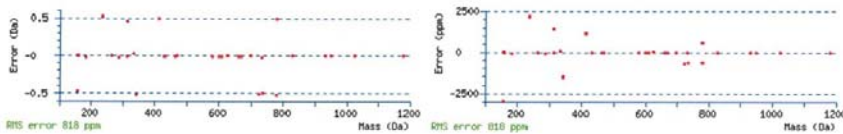
Peptide View

MS/MS Fragmentation of **SPERRPVEIRPATAA**
Found in **00036 Syngenta**, SyngentaMatch to Query 101: 1648.874628 from (825.444590,2+) intensity (11496.0000)
Title: 12: Sum of 9 scans in range 1116 (rt=15.7627, f=3, i=55) to 1140 (rt=15.9727, f=3, i=63) [D:\Syngenta 23099\99570Asp.raw]
Data file D:\Syngenta 23099\99570Asp.raw

Click mouse within plot area to zoom in by factor of two about that point

Or, to Da Monoisotopic mass of neutral peptide Mr(calc): 1648.9009
Fixed modifications: Carboxymethyl (C)
Ions Score: 29 Expect: 0.00028
Matches (Bold Red): 32/146 fragment ions using 68 most intense peaks

#	a	a ⁺⁺	a ⁺	a ⁺⁺⁺	b	b ⁺⁺	b ⁺	b ⁺⁺⁺	Seq.	y	y ⁺⁺	y ⁺	y ⁺⁺⁺	#
1	60.0444	30.5258			88.0393	44.5233			S					15
2	157.0972	79.0522			185.0921	93.0497			P	1562.8762	781.9417	1545.8496	773.4284	14
3	286.1397	143.5735			314.1347	157.5710			E	1465.8234	733.4153	1448.7968	724.9021	13
4	442.2409	221.6241	425.2143	213.1108	470.2358	235.6215	453.2092	227.1082	R	1336.7808	668.8940	1319.7542	660.3808	12
5	598.3420	299.6746	581.3154	291.1613	626.3369	313.6721	609.3103	305.1588	R	1180.6797	590.8435	1163.6531	582.3302	11
6	695.3947	348.2010	678.3682	339.6877	723.3896	362.1985	706.3631	353.6852	P	1024.5786	512.7929	1007.5520	504.2796	10
7	794.4631	397.7352	777.4366	389.2219	822.4581	411.7327	805.4315	403.2194	V	927.5258	464.2665	910.4993	455.7533	9
8	923.5057	462.2565	906.4792	453.7432	951.5007	476.2540	934.4741	467.7407	E	828.4574	414.7323	811.4308	406.2191	8
9	1036.5898	518.7985	1019.5633	510.2853	1064.5847	532.7960	1047.5582	524.2827	I	699.4148	350.2110	682.3883	341.6978	7
10	1192.6909	596.8491	1175.6644	588.3358	1220.6858	610.8466	1203.6593	602.3333	R	586.3307	293.6690	569.3042	285.1557	6
11	1289.7437	645.3755	1272.7171	636.8622	1317.7386	659.3729	1300.7120	650.8597	P	430.2296	215.6185			5
12	1360.7808	680.8940	1343.7542	672.3808	1388.7757	694.8915	1371.7492	686.3782	A	333.1769	167.0921			4
13	1461.8285	731.4179	1444.8019	722.9046	1489.8234	745.4153	1472.7968	736.9021	T	262.1397	131.5735			3
14	1532.8656	766.9364	1515.8390	758.4232	1560.8605	780.9339	1543.8340	772.4206	A	161.0921	81.0497			2
15									A	90.0550	45.5311			1

NCBI BLAST search of **SPERRPVEIRPATAA**
(Parameters: blastp, nr protein database, expect=20000, no filter, PAM30)
Other BLAST [web gateways](#)

All matches to this query

Score	Mr(calc)	Delta	Sequence
28.6	1648.9009	-0.0262	SPERRPVEIRPATAA

Mascot: <http://www.matrixscience.com/>

mhtml:file:///K:\Syngenta Mascot Exports\AspN 2.mht

29/03/2012

Mascot Search Results: Peptide View

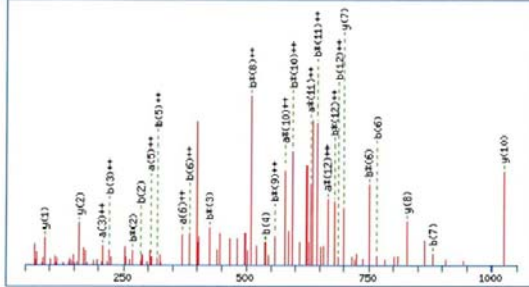
Page 1 of 1

**[MATRIX]
(SCIENCE)** Mascot Search Results

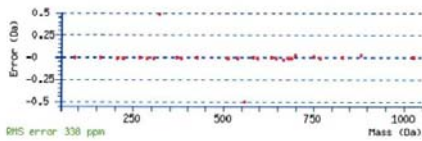
Peptide View

MS/MS Fragmentation of **ERRPVEIRPATAA**
Found in **00036 Syngenta**, SyngentaMatch to Query 72: 1464.762912 from (489.261580.3+) intensity (5418.0000)
Title: 8: Sum of 9 scans in range 1015 (rt=14.6573, f=2, i=177) to 1023 (rt=14.7273, f=2, i=185) [D:\Syngenta 23099\99570\Asp.raw]
Data file D:\Syngenta 23099\99570\Asp.raw

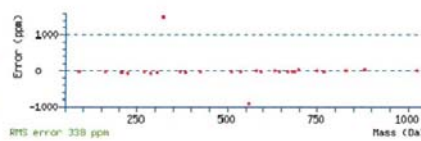
Click mouse within plot area to zoom in by factor of two about that point

Or, 50 1050 Da Monoisotopic mass of neutral peptide Mr(calc): 1464.8161
Fixed modifications: Carboxymethyl (C)
Ions Score: 20 Expect: 0.0025
Matches (Bold Red): 27/130 fragment ions using 58 most intense peaks

#	a	a++	a*	a*++	b	b++	b*	b*++	Seq.	y	y++	y*	y*++	#
1	102.0550	51.5311			130.0499	65.5286			E					13
2	258.1561	129.5817	241.1295	121.0684	286.1510	143.5791	269.1244	135.0659	R	1336.7808	668.8940	1319.7542	660.3808	12
3	414.2572	207.6322	397.2306	199.1190	442.2521	221.6297	425.2255	213.1164	R	1180.6797	590.8435	1163.6531	582.3302	11
4	511.3099	256.1586	494.2834	247.6453	539.3049	270.1561	522.2783	261.6428	P	1024.5786	512.7929	1007.5520	504.2796	10
5	610.3784	305.6928	593.3518	297.1795	638.3733	319.6903	621.3467	311.1770	V	927.5258	464.2665	910.4993	455.7533	9
6	739.4209	370.2141	722.3944	361.7008	767.4159	384.2116	750.3893	375.6983	E	828.4574	414.7323	811.4308	406.2191	8
7	852.5050	426.7561	835.4785	418.2429	880.4999	440.7536	863.4734	432.2403	I	699.4148	350.2110	682.3883	341.6978	7
8	1008.6061	504.8067	991.5796	496.2934	1036.6010	518.8042	1019.5745	510.2909	R	586.3307	293.6690	569.3042	285.1557	6
9	1105.6589	553.3331	1088.6323	544.8198	1133.6538	567.3305	1116.6273	558.8173	P	430.2296	215.6185			5
10	1176.6960	588.8516	1159.6695	580.3384	1204.6909	602.8491	1187.6644	594.3358	A	333.1769	167.0921			4
11	1277.7437	639.3755	1260.7171	630.8622	1305.7386	653.3729	1288.7120	644.8597	T	262.1397	131.5735			3
12	1348.7808	674.8940	1331.7542	666.3808	1376.7757	688.8915	1359.7492	680.3782	A	161.0921	81.0497			2
13									A	90.0550	45.5311			1



RMS error: 338 ppm



RMS error: 338 ppm

NCBI BLAST search of **ERRPVEIRPATAA**
(Parameters: blastp, nr protein database, expect=20000, no filter, PAM30)
Other BLAST [web gateways](#)

All matches to this query

Score	Mr(calc):	Delta	Sequence
19.9	1464.8161	-0.0532	ERRPVEIRPATAA

Mascot: <http://www.matrixscience.com/>

mhtml:file://K:\Syngenta Mascot Exports\AspN 3.mht

29/03/2012

Mascot Search Results: Peptide View

Page 1 of 2

Mascot Search Results

Peptide View

MS/MS Fragmentation of **DIVNHYI**

Found in **00036 Syngenta**, Syngenta

Match to Query 29: 872.441684 from(873.448960,1+) intensity(2791.0000)

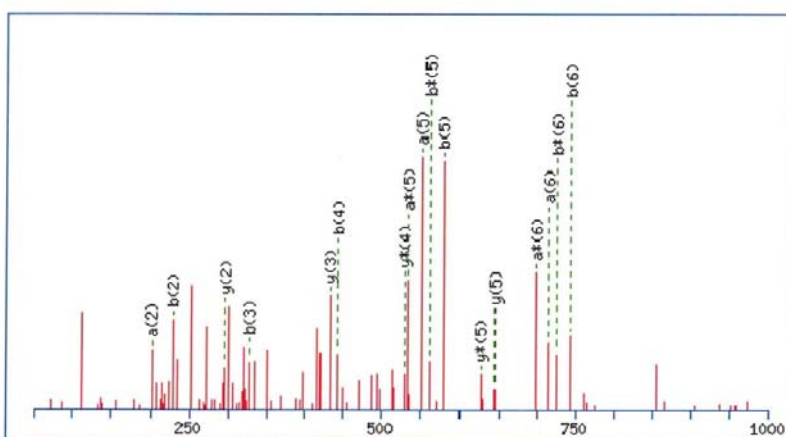
Title: 64: Sum of 9 scans in range 2371 (rt=30.0307, f=2, i=650) to 2379 (rt=30.1007, f=2, i=658)

[D:\Syngenta 23099\99570Asp.raw]

Data file D:\Syngenta 23099\99570Asp.raw

Click mouse within plot area to zoom in by factor of two about that point

Or, 50 1000



Monoisotopic mass of neutral peptide Mr(calc): 872.4392

Fixed modifications: Carboxymethyl (C)

Ions Score: 19 Expect: 0.0013

Matches (**Bold Red**): 17/27 fragment ions using 50 most intense peaks

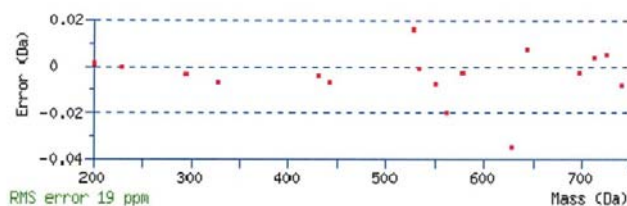
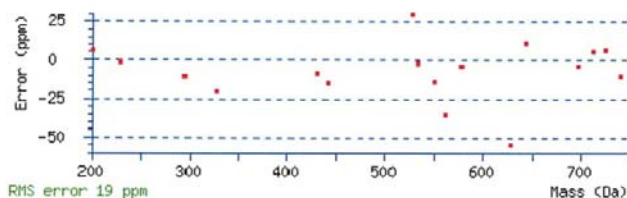
#	a	a*	b	b*	Seq.	y	y*	#
1	88.0393		116.0342		D			7
2	201.1234		229.1183		I	758.4196	741.3930	6
3	300.1918		328.1867		V	645.3355	628.3089	5
4	414.2347	397.2082	442.2296	425.2031	N	546.2671	529.2405	4
5	551.2936	534.2671	579.2885	562.2620	H	432.2241		3
6	714.3570	697.3304	742.3519	725.3253	Y	295.1652		2
7					I	132.1019		1

mhtml:file://K:\Syngenta Mascot Exports\AspN 4.mht

29/03/2012

Mascot Search Results: Peptide View

Page 2 of 2

532
29/3/12NCBI BLAST search of [DIVNHYL](#)

(Parameters: blastp, nr protein database, expect=20000, no filter, PAM30)

Other BLAST [web gateways](#)

All matches to this query

Score	Mr(calc):	Delta	Sequence
19.0	872.4392	0.0025	DIVNHYL

Mascot: <http://www.matrixscience.com/>

Mascot Search Results: Peptide View

Page 1 of 2

Mascot Search Results

55~
21/3/12

Peptide View

MS/MS Fragmentation of **DDLRLQ**Found in **00036 Syngenta**, Syngenta

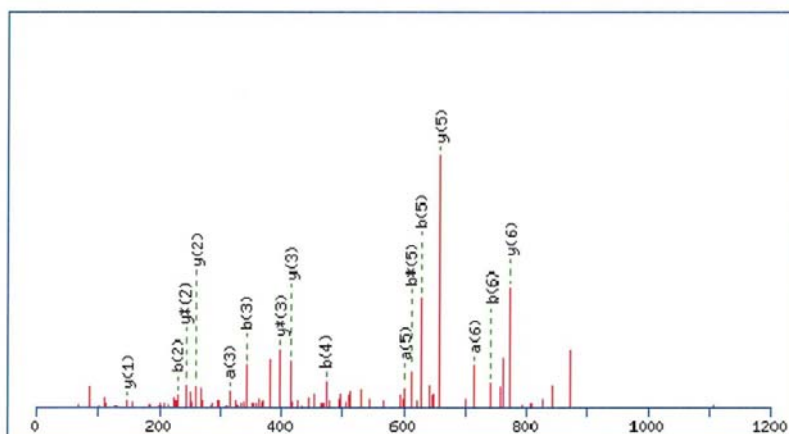
Match to Query 30: 887.429984 from(888.437260,1+) intensity(8959.0000)

Title: 31: Sum of 9 scans in range 1420 (rt=19.1911, f=2, i=316) to 1428 (rt=19.2611, f=2, i=324)

[D:\Syngenta 23099\99570Asp.raw]

Data file D:\Syngenta 23099\99570Asp.raw

Click mouse within plot area to zoom in by factor of two about that point

Or, 0 1200 Da 

Monoisotopic mass of neutral peptide Mr(calc): 887.4348

Fixed modifications: Carboxymethyl (C)

Ions Score: 33 Expect: 2.5e-005

Matches (**Bold Red**): 16/28 fragment ions using 28 most intense peaks

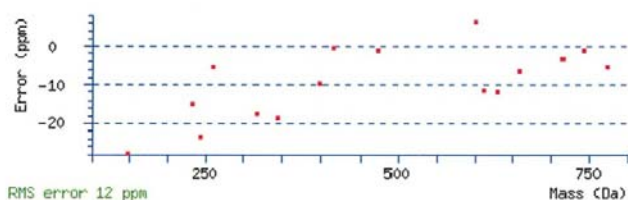
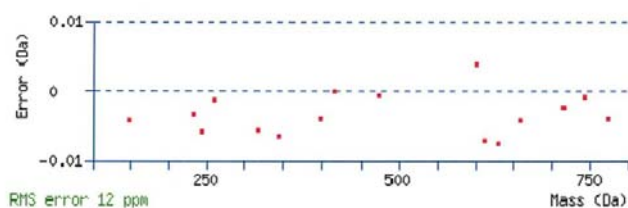
#	a	a*	b	b*	Seq.	y	y*	#
1	88.0393		116.0342		D			7
2	203.0662		231.0612		D	773.4152	756.3886	6
3	316.1503		344.1452		L	658.3883	641.3617	5
4	445.1929		473.1878		E	545.3042	528.2776	4
5	601.2940	584.2675	629.2889	612.2624	R	416.2616	399.2350	3
6	714.3781	697.3515	742.3730	725.3464	L	260.1605	243.1339	2
7					Q	147.0764	130.0499	1

mhtml:file://K:\Syngenta Mascot Exports\AspN 5.mht

29/03/2012

Mascot Search Results: Peptide View

Page 2 of 2

NCBI **BLAST** search of [DDLRLQ](#)

(Parameters: blastp, nr protein database, expect=20000, no filter, PAM30)

Other BLAST [web gateways](#)

All matches to this query

Score	Mr(calc):	Delta	Sequence
33.0	887.4348	-0.0049	DDLRLQ

Mascot: <http://www.matrixscience.com/>

Mascot Search Results: Peptide View

Page 1 of 2

Mascot Search Results

532
21/3/12

Peptide View

MS/MS Fragmentation of **DLERLQ**Found in **00036 Syngenta**, Syngenta

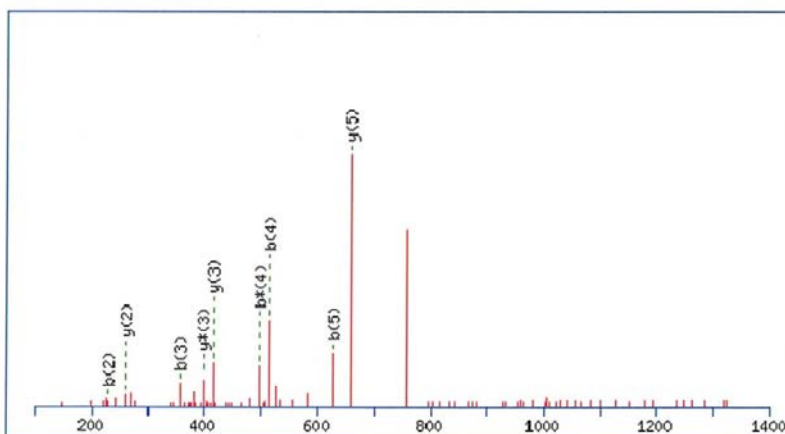
Match to Query 6: 772.404554 from(773.411830,1+) intensity(2943.0000)

Title: 20: Sum of 9 scans in range 1294 (rt=17.7752, f=2, i=267) to 1302 (rt=17.8452, f=2, i=275)

[D:\Syngenta 23099\99570Asp.raw]

Data file D:\Syngenta 23099\99570Asp.raw

Click mouse within plot area to zoom in by factor of two about that point

Or, 100 1400 

Monoisotopic mass of neutral peptide Mr(calc): 772.4079

Fixed modifications: Carboxymethyl (C)

Ions Score: 28 Expect: 0.00023

Matches (**Bold Red**): 9/24 fragment ions using 18 most intense peaks

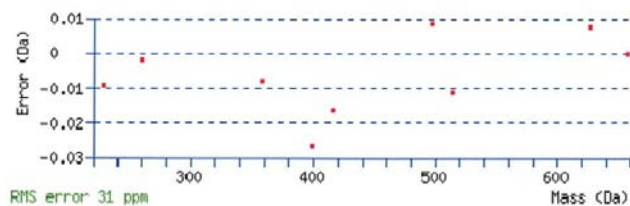
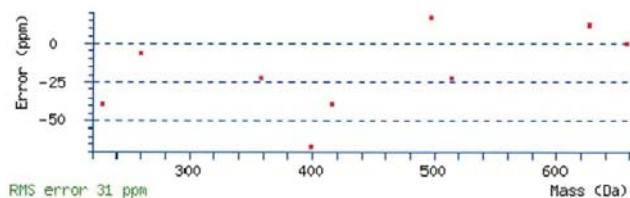
#	a	a*	b	b*	Seq.	y	y*	#
1	88.0393		116.0342		D			6
2	201.1234		229.1183		L	658.3883	641.3617	5
3	330.1660		358.1609		E	545.3042	528.2776	4
4	486.2671	469.2405	514.2620	497.2354	R	416.2616	399.2350	3
5	599.3511	582.3246	627.3461	610.3195	L	260.1605	243.1339	2
6					Q	147.0764	130.0499	1

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29/03/2012

Mascot Search Results: Peptide View

Page 2 of 2

S37
29/3/12NCBI **BLAST** search of [DLERLQ](#)

(Parameters: blastp, nr protein database, expect=20000, no filter, PAM30)

Other BLAST [web gateways](#)

All matches to this query

Score	Mr(calc):	Delta	Sequence
28.2	772.4079	-0.0033	DLERLQ

Mascot: <http://www.matrixscience.com/>

Mascot Search Results: Peptide View

Page 1 of 2

Mascot Search Results

 53-
29/3/12

Peptide View

MS/MS Fragmentation of **DLERLQ**Found in **00036 Syngenta**, Syngenta

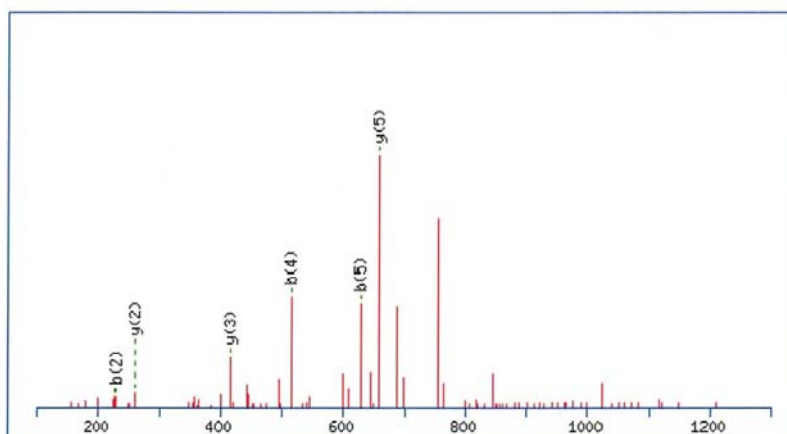
Match to Query 7: 772.406844 from(773.414120,1+) intensity(3882.0000)

Title: 57: Sum of 9 scans in range 1981 (rt=25.2949, f=6, i=76) to 2021 (rt=25.6448, f=6, i=84)

[D:\Syngenta 23099\99570Asp.raw]

Data file D:\Syngenta 23099\99570Asp.raw

Click mouse within plot area to zoom in by factor of two about that point

Or, 100 1300 

Monoisotopic mass of neutral peptide Mr(calc): 772.4079

Fixed modifications: Carboxymethyl (C)

Ions Score: 35 Expect: 5.3e-005

Matches (**Bold Red**): 6/24 fragment ions using 11 most intense peaks

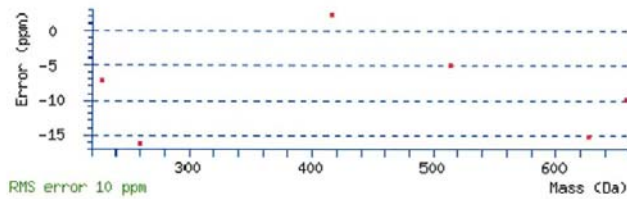
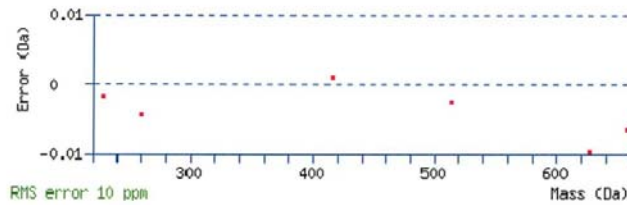
#	a	a*	b	b*	Seq.	y	y*	#
1	88.0393		116.0342		D			6
2	201.1234		229.1183		L	658.3883	641.3617	5
3	330.1660		358.1609		E	545.3042	528.2776	4
4	486.2671	469.2405	514.2620	497.2354	R	416.2616	399.2350	3
5	599.3511	582.3246	627.3461	610.3195	L	260.1605	243.1339	2
6					Q	147.0764	130.0499	1

mhtml:file://K:\Syngenta Mascot Exports\AspN 7.mht

29/03/2012

Mascot Search Results: Peptide View

Page 2 of 2

NCBI BLAST search of [DLERLQ](#)

(Parameters: blastp, nr protein database, expect=20000, no filter, PAM30)

Other BLAST [web gateways](#)

All matches to this query

Score	Mr(calc):	Delta	Sequence
34.5	772.4079	-0.0011	DLERLQ

Mascot: <http://www.matrixscience.com/>

Mascot Search Results: Peptide View

Page 1 of 2

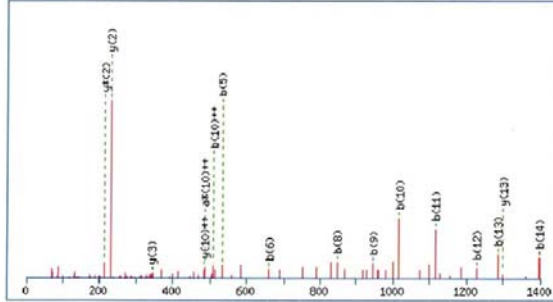
Mascot Search Results

Peptide View

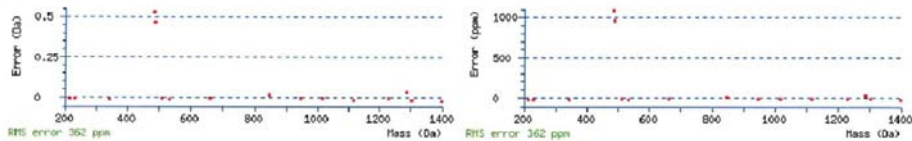
MS/MS Fragmentation of **EAQGFKSVVAVIGLPN**
Found in **00036 Syngenta**, SyngentaMatch to Query 99: 1627.880548 from(814.947550,2+) intensity(4671.0000)
Title: 94: Sum of 9 scans in range 3736 (rt=46.952, f=2, i=1125) to 3752 (rt=47.0919, f=2, i=1133) [D:\Syngenta 23099\99570Asp.raw]
Data file D:\Syngenta 23099\99570Asp.raw

Click mouse within plot area to zoom in by factor of two about that point

Or, Plot from 0 to 1400 Da Full range

Monoisotopic mass of neutral peptide Mr(calc): 1627.8934
Fixed modifications: Carboxymethyl (C)
Ions Score: 53 Expect: 1.9e-006
Matches (Bold Red): 16/172 fragment ions using 31 most intense peaks

#	a	a++	a*	a***	b	b++	b*	b***	Seq.	y	y++	y*	y***	#
1	102.0550	51.5311			130.0499	65.5286			E					16
2	173.0921	87.0497			201.0870	101.0471			A	1499.8580	750.4327	1482.8315	741.9194	15
3	301.1506	151.0790	284.1241	142.5657	329.1456	165.0764	312.1190	156.5631	Q	1428.8209	714.9141	1411.7944	706.4008	14
4	358.1721	179.5897	341.1456	171.0764	386.1670	193.5872	369.1405	185.0739	G	1300.7623	650.8848	1283.7358	642.3715	13
5	505.2405	253.1239	488.2140	244.6106	533.2354	267.1214	516.2089	258.6081	F	1243.7409	622.3741	1226.7143	613.8608	12
6	633.3355	317.1714	616.3089	308.6581	661.3304	331.1688	644.3039	322.6556	K	1096.6725	548.8399	1079.6459	540.3266	11
7	720.3675	360.6874	703.3410	352.1741	748.3624	374.6849	731.3359	366.1716	S	968.5775	484.7924	951.5510	476.2791	10
8	819.4359	410.2216	802.4094	401.7083	847.4308	424.2191	830.4043	415.7058	V	881.5455	441.2764	864.5189	432.7631	9
9	918.5043	459.7558	901.4778	451.2425	946.4993	473.7533	929.4727	465.2400	V	782.4771	391.7422	765.4505	383.2289	8
10	989.5415	495.2744	972.5149	486.7611	1017.5364	509.2718	1000.5098	500.7585	A	683.4087	342.2080	666.3821	333.6947	7
11	1088.6099	544.8086	1071.5833	536.2953	1116.6048	558.8060	1099.5782	550.2928	V	612.3715	306.6894	595.3450	298.1761	6
12	1201.6939	601.3506	1184.6674	592.8373	1229.6888	615.3481	1212.6623	606.8348	I	513.3031	257.1552	496.2766	248.6419	5
13	1258.7154	629.8613	1241.6888	621.3481	1286.7103	643.8588	1269.6838	635.3455	G	400.2191	200.6132	383.1925	192.0999	4
14	1371.7995	686.4034	1354.7729	677.8901	1399.7944	700.4008	1382.7678	691.8876	L	343.1976	172.1024	326.1710	163.5892	3
15	1468.8522	734.9298	1451.8257	726.4165	1496.8471	748.9272	1479.8206	740.4139	P	230.1135	115.5604	213.0870	107.0471	2
16									N	133.0608	67.0340	116.0342	58.5207	1

NCBI BLAST search of **EAQGFKSVVAVIGLPN**
(Parameters: blastp, nr protein database, expect=20000, no filter, PAM30)
Other BLAST [web gateways](#)

All matches to this query



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29/03/2012

Mascot Search Results: Peptide View

Page 2 of 2

Score	Mr(calc)	Delta	Sequence
52.6	1627.8934	-0.0128	EAQGFKSVVAVIGLPN

572
29/3/12Mascot: <http://www.matrixscience.com/>

mhtml:file://K:\Syngenta Mascot Exports\AspN 8.mht

29/03/2012

Mascot Search Results: Peptide View

Page 1 of 2

{MATRIX} {SCIENCE} Mascot Search Results

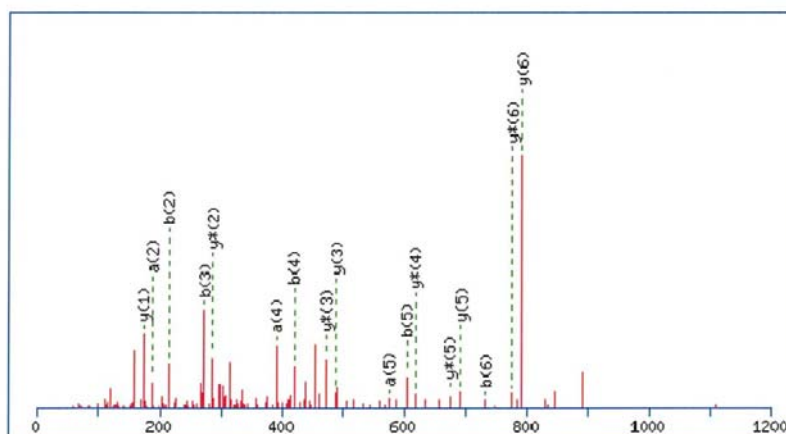
530
27/3/12

Peptide View

MS/MS Fragmentation of **DVGFWQR**
Found in **00036 Syngenta**, Syngenta

Match to Query 31: 906.435354 from(907.442630,1+) intensity(9974.0000)
Title: 65: Sum of 9 scans in range 2396 (rt=30.2911, f=4, i=147) to 2428 (rt=30.5711, f=4, i=155)
[D:\Syngenta 23099\99570Asp.raw]
Data file D:\Syngenta 23099\99570Asp.raw

Click mouse within plot area to zoom in by factor of two about that point

Or, 0 1200 

Monoisotopic mass of neutral peptide Mr(calc): 906.4348
Fixed modifications: Carboxymethyl (C)
Ions Score: 36 Expect: 1.3e-005
Matches (**Bold Red**): 17/26 fragment ions using 25 most intense peaks

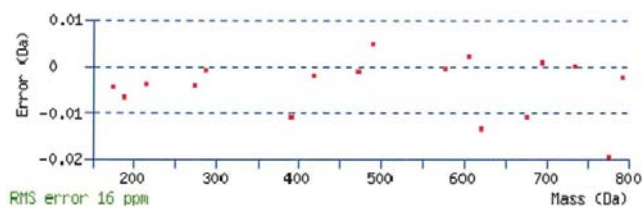
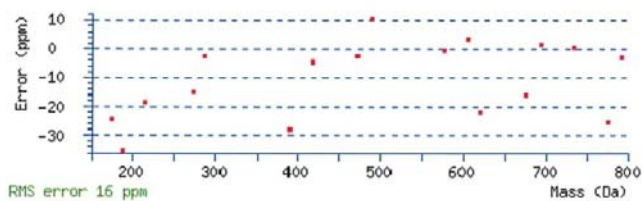
#	a	a*	b	b*	Seq.	y	y*	#
1	88.0393		116.0342		D			7
2	187.1077		215.1026		V	792.4151	775.3886	6
3	244.1292		272.1241		G	693.3467	676.3202	5
4	391.1976		419.1925		F	636.3253	619.2987	4
5	577.2769		605.2718		W	489.2568	472.2303	3
6	705.3355	688.3089	733.3304	716.3039	Q	303.1775	286.1510	2
7					R	175.1190	158.0924	1

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29/03/2012

Mascot Search Results: Peptide View

Page 2 of 2

SJW
29/3/12NCBI **BLAST** search of [DVGFWQR](#)

(Parameters: blastp, nr protein database, expect=20000, no filter, PAM30)

Other BLAST [web gateways](#)

All matches to this query

Score	Mr(calc):	Delta	Sequence
35.9	906.4348	0.0005	DVGFWQR

Mascot: <http://www.matrixscience.com/>

Mascot Search Results: Peptide View

Page 1 of 2

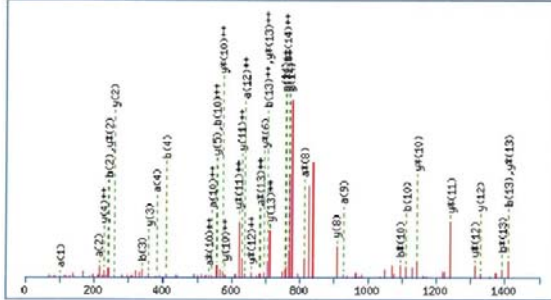
(MATRIX)
(SCIENCE) **Mascot Search Results**

Peptide View

MS/MS Fragmentation of **ELPAPPRPVRPVTQI**
Found in **00036 Syngenta**, Syngenta

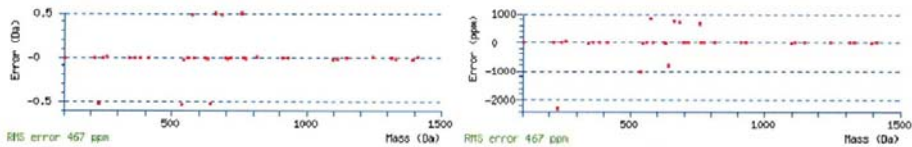
Match to Query 102: 1668.951848 from(835.483200,2+) intensity(14996.0000)
Title: 72: Sum of 9 scans in range 2566 (rt=32.1277, f=5, i=134) to 2606 (rt=32.4778, f=5, i=142) [D:\Syngenta 23099\99570Asp.raw]
Data file D:\Syngenta 23099\99570Asp.raw

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from 0 to 1500 Da Full range



Monoisotopic mass of neutral peptide Mr(calc): 1668.9675
Fixed modifications: Carboxymethyl (C)
Ions Score: 22 Expect: 0.0029
Matches (Bold Red): 42/142 fragment ions using 85 most intense peaks

#	a	a ⁺⁺	a ⁺	a ⁺⁺⁺	b	b ⁺⁺	b ⁺	b ⁺⁺⁺	Seq.	y	y ⁺⁺	y ⁺	y ⁺⁺⁺	#
1	102.0550	51.5311			130.0499	65.5286			E					15
2	215.1390	108.0731			243.1339	122.0706			L	1540.9322	770.9697	1523.9057	762.4565	14
3	312.1918	156.5995			340.1867	170.5970			P	1427.8481	714.4277	1410.8216	705.9144	13
4	383.2289	192.1181			411.2238	206.1155			A	1330.7954	665.9013	1313.7688	657.3881	12
5	480.2817	240.6445			508.2766	254.6419			P	1259.7583	630.3828	1242.7317	621.8695	11
6	577.3344	289.1709			605.3293	303.1683			P	1162.7055	581.8564	1145.6790	573.3431	10
7	733.4355	367.2214	716.4090	358.7081	761.4305	381.2189	744.4039	372.7056	R	1065.6527	533.3300	1048.6262	524.8167	9
8	830.4883	415.7478	813.4618	407.2345	858.4832	429.7452	841.4567	421.2320	P	909.5516	455.2795	892.5251	446.7662	8
9	929.5567	465.2820	912.5302	456.7687	957.5516	479.2795	940.5251	470.7662	V	812.4989	406.7531	795.4723	398.2398	7
10	1085.6578	543.3326	1068.6313	534.8193	1113.6527	557.3300	1096.6262	548.8167	R	713.4305	357.2189	696.4039	348.7056	6
11	1182.7106	591.8589	1165.6840	583.3457	1210.7055	605.8564	1193.6790	597.3431	P	557.3293	279.1683	540.3028	270.6550	5
12	1281.7790	641.3931	1264.7525	632.8799	1309.7739	655.3906	1292.7474	646.8773	V	460.2766	230.6419	443.2500	222.1287	4
13	1382.8267	691.9170	1365.8001	683.4037	1410.8216	705.9144	1393.7950	697.4012	T	361.2082	181.1077	344.1816	172.5944	3
14	1510.8853	755.9463	1493.8587	747.4330	1538.8802	769.9437	1521.8536	761.4305	Q	260.1605	130.5839	243.1339	122.0706	2
15									I	132.1019	66.5546			1



NCBI BLAST search of **ELPAPPRPVRPVTQI**
(Parameters: blastn, nr protein database, expect=20000, no filter, PAM30)
Other BLAST [web gateways](#)

All matches to this query

Score	Mr(calc)	Delta	Sequence
-------	----------	-------	----------

mhtml:file://K:\Syngenta Mascot Exports\AspN 10.mht

29/03/2012

Mascot Search Results: Peptide View

Page 2 of 2

22.3	1668.9675	-0.0157	ELPAPPRPVRPVTQI
3.7	1669.7294	-0.7775	MPKINSFNYPNPVN
3.6	1669.7294	-0.7775	MPKINSFNYPNPVN
3.5	1669.7294	-0.7775	MPKINSFNYPNPVN
3.3	1667.7746	1.1772	EKTPVSEKVTCCCT
2.3	1668.7453	0.2065	MPKINSFNYPNPVN
1.5	1669.7294	-0.7775	MPKINSFNYPNPVN

SJW
29/3/12Mascot: <http://www.matrixscience.com/>

mhtml:file:///K:\Syngenta Mascot Exports\AspN 10.mht

29/03/2012

Mascot Search Results: Protein View

Page 1 of 1

Mascot Search Results

Protein View

Match to: 00036 Syngenta Score: 66

Syngenta

Found in search of D:\Syngenta 23099\99570Chy.raw

Nominal mass (M_r): 20663; Calculated pI value: 6.37

NCBI BLAST search of 00036 Syngenta against nr

Unformatted [sequence string](#) for pasting into other applications

Fixed modifications: Carboxymethyl (C)

Variable modifications: Deamidated (NQ), Oxidation (M)

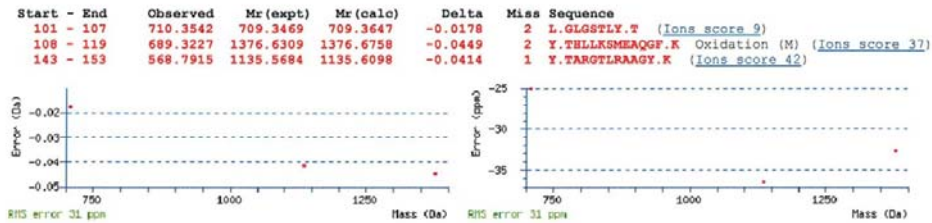
Cleavage by Chymotrypsin: cuts C-term side of FYWL unless next residue is P

Sequence Coverage: 16%

Matched peptides shown in **Bold Red**

1 MSPERRPVEI RPATAADMAA VCDIVNHIE TSTVNFRTPE QTPQEWIDDL
51 ERLQDRYPWL VAEVEGVVAG IAYAGPWKAR NAYDWTVEST VYVSHRHQRL
101 **GLGSTLYTHL LKSMKAQGF** SVVAVIGLPN DPSVRLHEAL GYTARGLRA
151 **AGYKHGGWHD VGFWRDFEL PAPPRPVRPV** TQI

Show predicted peptides also

Sort Peptides By ☒ Residue Number ☐ Increasing Mass ☐ Decreasing MassMascot: <http://www.matrixscience.com/>

mhtml:file://K:\Syngenta Mascot Exports\Chy Summary.mht

29/03/2012

Mascot Search Results: Peptide View

Page 1 of 2

Mascot Search Results

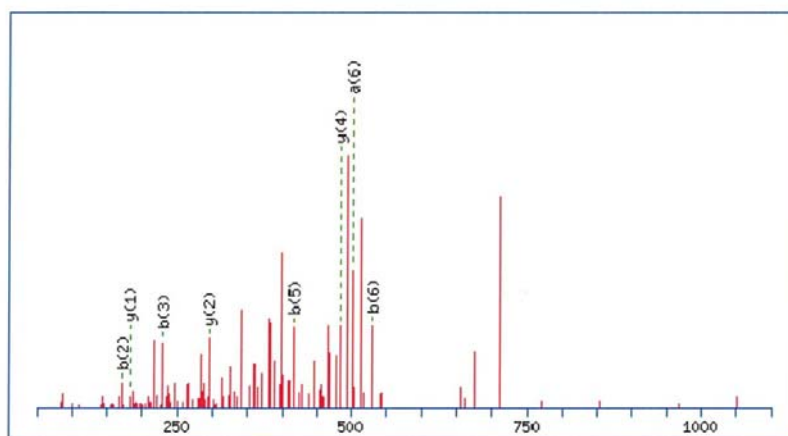
Peptide View

MS/MS Fragmentation of **GLGSTLY**
Found in **00036 Syngenta**, Syngenta

Match to Query 1: 709.346904 from(710.354180,1+) intensity(5097.0000)
Title: 25: Sum of 9 scans in range 2021 (rt=28.7165, f=2, i=413) to 2029 (rt=28.7865, f=2, i=421)
[D:\Syngenta 23099\99570Chy.raw]
Data file D:\Syngenta 23099\99570Chy.raw

Click mouse within plot area to zoom in by factor of two about that point

Or, 50 1100



Monoisotopic mass of neutral peptide Mr(calc): 709.3647
Fixed modifications: Carboxymethyl (C)
Ions Score: 9 Expect: 0.011
Matches (**Bold Red**): 8/18 fragment ions using 28 most intense peaks

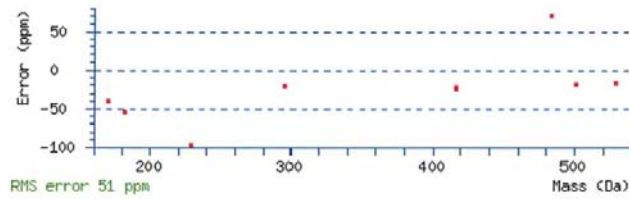
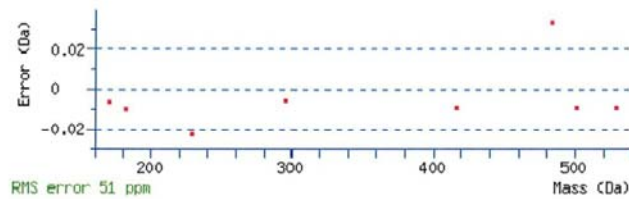
#	a	b	Seq.	y	#
1	30.0338	58.0287	G		7
2	143.1179	171.1128	L	653.3505	6
3	200.1394	228.1343	G	540.2664	5
4	287.1714	315.1663	S	483.2449	4
5	388.2191	416.2140	T	396.2129	3
6	501.3031	529.2980	L	295.1652	2
7			Y	182.0812	1

mhtml:file://K:\Syngenta Mascot Exports\Chy 1.mht

29/03/2012

Mascot Search Results: Peptide View

Page 2 of 2

NCBI **BLAST** search of [GLGSTLY](#)

(Parameters: blastp, nr protein database, expect=20000, no filter, PAM30)

Other BLAST [web gateways](#)

All matches to this query

Score	Mr(calc):	Delta	Sequence
9.4	709.3647	-0.0178	GLGSTLY

Mascot: <http://www.matrixscience.com/>

Mascot Search Results: Peptide View

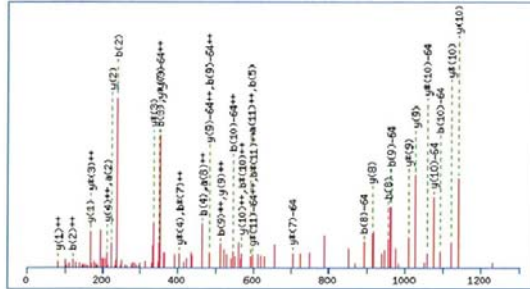
Page 1 of 1

(MATRIX) Mascot Search Results**Peptide View**

MS/MS Fragmentation of **THLLKSMEAQGF**
 Found in **00036 Syngenta**, Syngenta

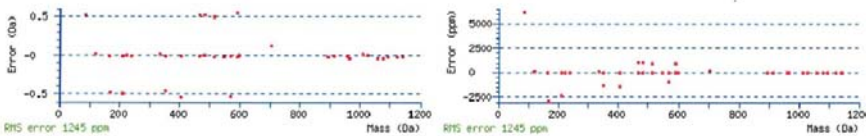
Match to Query 47: 1376.630928 from (689.322740, 2+) intensity (7151.0000)
 Title: 14: Sum of 9 scans in range 1417 (rt=20.4923, f=2, i=240) to 1425 (rt=20.5623, f=2, i=248) [D:\Syngenta 23099\99570Chy.raw]
 Data file D:\Syngenta 23099\99570Chy.raw

Click mouse within plot area to zoom in by factor of two about that point
 Or, Plot from 0 to 1300 Da Full range



Monoisotopic mass of neutral peptide Mr(calc): 1376.6758
 Fixed modifications: Carboxymethyl (C)
 Variable modifications:
 M7 : Oxidation (M), with neutral losses 63.9983 (shown in table), 0.0000
 Ions Score: 37 Expect: 2.1e-005
 Matches (Bold Red): 39/176 fragment ions using 50 most intense peaks

#	a	a ⁺⁺	a [*]	a ⁺⁺⁺	b	b ⁺⁺	b [*]	b ⁺⁺⁺	Seq.	y	y ⁺⁺	y [*]	y ⁺⁺⁺	#
1	74.0600	37.5337			102.0550	51.5311			T					12
2	211.1190	106.0631			239.1139	120.0606			H	1212.6372	606.8222	1195.6106	598.3089	11
3	324.2030	162.6051			352.1979	176.6026			L	1075.5782	538.2928	1058.5517	529.7795	10
4	437.2871	219.1472			465.2820	233.1446			L	962.4942	481.7507	945.4676	473.2375	9
5	565.3820	283.1947	548.3555	274.6814	593.3770	297.1921	576.3504	288.6788	K	849.4101	425.2087	832.3836	416.6954	8
6	652.4141	326.7107	635.3875	318.1974	680.4090	340.7081	663.3824	332.1949	S	721.3152	361.1612	704.2886	352.6479	7
7	735.4512	368.2292	718.4246	359.7160	763.4461	382.2267	746.4196	373.7134	M	634.2831	317.6452	617.2566	309.1319	6
8	864.4938	432.7505	847.4672	424.2373	892.4887	446.7480	875.4621	438.2347	E	551.2460	276.1266	534.2195	267.6134	5
9	935.5309	468.2691	918.5043	459.7558	963.5258	482.2665	946.4993	473.7533	A	422.2034	211.6053	405.1769	203.0921	4
10	1063.5895	532.2984	1046.5629	523.7851	1091.5844	546.2958	1074.5578	537.7826	Q	351.1663	176.0868	334.1397	167.5735	3
11	1120.6109	560.8091	1103.5844	552.2958	1148.6059	574.8066	1131.5793	566.2933	G	223.1077	112.0575			2
12									F	166.0863	83.5468			1



NCBI BLAST search of **THLLKSMEAQGF**
 (Parameters: blastp, nr protein database, expect=20000, no filter, PAM30)
 Other BLAST [web gateways](#)

All matches to this query

Score	Mr(calc)	Delta	Sequence
36.8	1376.6758	-0.0449	THLLKSMEAQGF

Mascot: <http://www.matrixscience.com/>

mhtml:file://K:\Syngenta Mascot Exports\Chy 2.mht

29/03/2012

Mascot Search Results: Peptide View

Page 2 of 2

(Parameters: blastp, nr protein database, expect=20000, no filter, PAM30)
Other BLAST [web gateways](#)

All matches to this query

Score	Mr(calc)	Delta	Sequence
42.1	1135.6098	-0.0414	TARGTLRAAGY

ST
29/3/12

Mascot: <http://www.matrixscience.com/>

mhtml:file:///K:/Syngenta Mascot Exports/Chy 3.mht

29/03/2012

10.3 Edman Sequencing Data for β -lactoglobulin standard

N-terminal sequencing data sheet

Client / Job number: Syngenta / 23099
 Sample number: beta-lactoglobulin
 Performed by/date: EPS 02 Apr 2012
 Reviewed by/date: _____

Residue Number	Sequence		Comments
	PTH-AA	pmoles	
1	L	5.4	
2	I	5.1	
3	V	4.6	
4	T	3.6	
5	Q	4.2	
6	T	3.1	
7	M	3.7	
8	K	3.6	
9	G	4.5	
10	L	4.3	
11	D	3.1	
12	I	3.9	
13	Q	3.6	
14	K	3.1	
15	V	3.4	
16			
17			
18			
19			
20			

* Calculation based on 10pmole containing standard.

Page 1 of 1

G:\Bio Chem\HomeDirs\Emilie\Projects\N-terminal sequencing\N-terminal sequencing data sheets\Data processing 1 seq.doc

SequencePro™

EPS 02 April 12
Job 220 23099
EPS 02 April 12

EPS
31 Jan 2012

SGS M-Scan Ltd
SGS M-Scan job: NA
SGS M-Scan sample: NA
Operator: EPS

SAMPLE INFORMATION

Sample Name: beta-Lactoglobulin
ID Code:

Std Amount: 10.000 pmols
Sample Amount: 10.000 pmols
Detector Scale: 0.005 AUFS

Comments:

SEQUENCER INFORMATION

Name: PROCISE
Method: Pulsed liquid
Operator: EPS

Model Number: 492
Cartridge: A


CHEMICAL INFORMATION

R1	1111174	22 March, 2012	X3	0	01 September, 2001
R2	1105087	06 March, 2012	PTH Column	B120608034	28 March, 2012
R3	1012198	23 March, 2012	Solvent A	1112630	25 March, 2012
R4	1111134	25 March, 2012	Solvent B	1107354	23 March, 2012
R5	1011047	30 March, 2012	Premix	1104103	02 March, 2012
S1	0	01 September, 2001	Guard Column	0	01 January, 2002
S2	1109711	26 March, 2012	Cartridge Seals	0	01 January, 2002
S3	1112502	26 March, 2012	Glass Fiber Filter	0	01 January, 2002
S4	1201170	28 March, 2012	PTH standards	1007028	01 January, 2002
X1	0	01 September, 2001	PTH standards	12345678	01 January, 2002
X2	0	01 September, 2001	Total Cycles Count		01 January, 2002

Saturday, March 31, 2012 11:25:07

120330_2A_bLG - Page 1 of 19

SequencePro™


 3/11/12

 SGS M-Scan Ltd
 SGS M-Scan job: NA
 SGS M-Scan sample: NA
 Operator: EPS

ORIGINAL METHOD TEMPLATE: C:\Program Files\AppliedBiosystems\ProCise\SequencePro\Methods\ProCise.met

CALIBRATION TABLE

COMPONENT	RTIME	RESPONSE	REFERENCE	INTERNAL STD	ABS WND	REL WND
Aspartic Acid	4.37	1.000	---	---	0.20	0.00
Asparagine	4.90	1.000	---	---	0.20	0.00
Serine	5.61	1.000	---	---	0.20	0.00
Glutamine	5.88	1.000	---	---	0.20	0.00
Threonine	6.18	1.000	---	---	0.20	0.00
Glycine	6.42	1.000	---	---	0.20	0.00
Glutamic Acid	6.88	1.000	---	---	0.20	0.00
Histidine	8.10	1.000	---	---	0.20	0.00
Alanine	8.84	1.000	---	---	0.20	0.00
Arginine	10.40	1.000	---	---	0.20	0.00
Tyrosine	11.15	1.000	---	---	0.20	0.00
Proline	13.52	1.000	---	---	0.20	0.00
Methionine	14.23	1.000	---	---	0.20	0.00
Valine	14.56	1.000	---	---	0.20	0.00
dptu	15.77	1.000	---	---	0.20	0.00
Tryptophan	16.83	1.000	---	---	0.20	0.00
Phenylalanine	17.52	1.000	---	---	0.20	0.00
Isoleucine	18.01	1.000	---	---	0.20	0.00
Lysine	18.25	1.000	---	---	0.20	0.00
Leucine	18.56	1.000	---	---	0.20	0.00

GLOBAL INTEGRATION EVENTS

EVENT	TIME	VALUE	EVENT	TIME	VALUE
Peak Detect Off	0.00	---	Valley to Valley Off	5.30	---
Valley to Valley On	3.90	---	Peak Detect Off	20.00	---
Peak Detect On	3.90	---			

INTEGRATION PARAMETERS

PEAK DETECTION PARAMETERS					
Bunching Factor	4		Noise Threshold:	0.954	µVolts
Max Peaks	128		Area Threshold:	41.000	µVolts
PEAK SEPARATION CRITERIA			EXPONENTIAL SKIM CRITERIA		
Width Ratio:	0.20		Peak Height Ratio:	5.00	
Valley to Peak Ratio:	0.01		Adjusted Height Ratio:	4.00	
Tangent Width:	1000.00		Valley Height Ratio:	3.00	

SEQUENCE CALLING PARAMETERS

Use Pmol Heights, Allow Negative Background Off, Refine Data On					
SEQUENCE SCORING VALUES					
Raw Slope 1:	2.00		Raw Yield	1.00	
Raw Slope 2:	1.00		Bkgd Yield	1.00	
Bkgd Slope 1:	2.00		Lag Yield	1.00	
Bkgd Slope 2:	1.00		Rep Yield	1.00	
Max Slope:	1.50		Low Yield	1.00	
Rule Book:	0.60		Bkgd Sensitivity	1.00	
Dev Mult:	3.00				

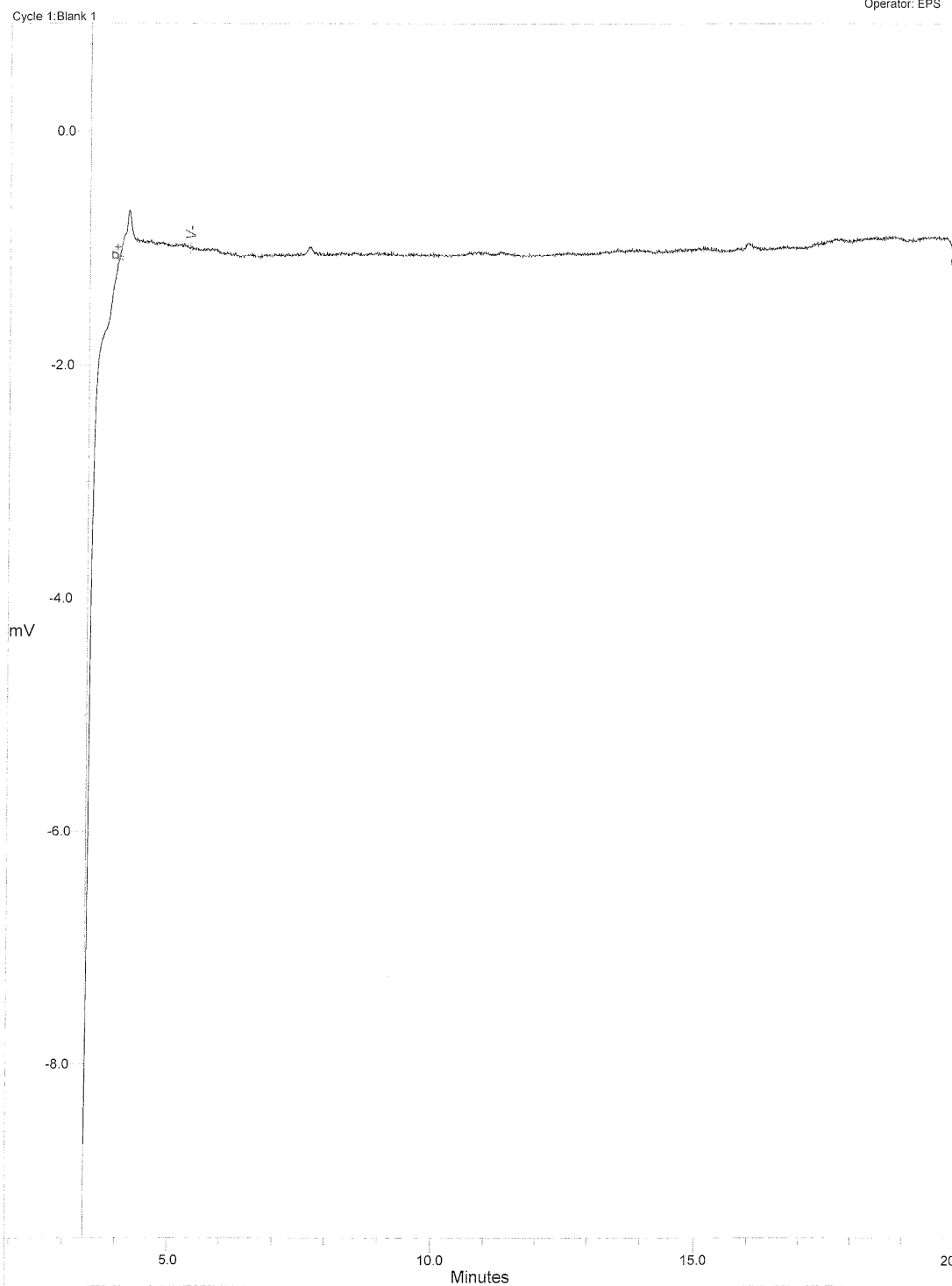
Saturday, March 31, 2012 11:25:07

120330_2A_bLG - Page 2 of 19

SequencePro™

EPS
3/19/2012

SGS M-Scan Ltd
SGS M-Scan job: NA
SGS M-Scan sample: NA
Operator: EPS



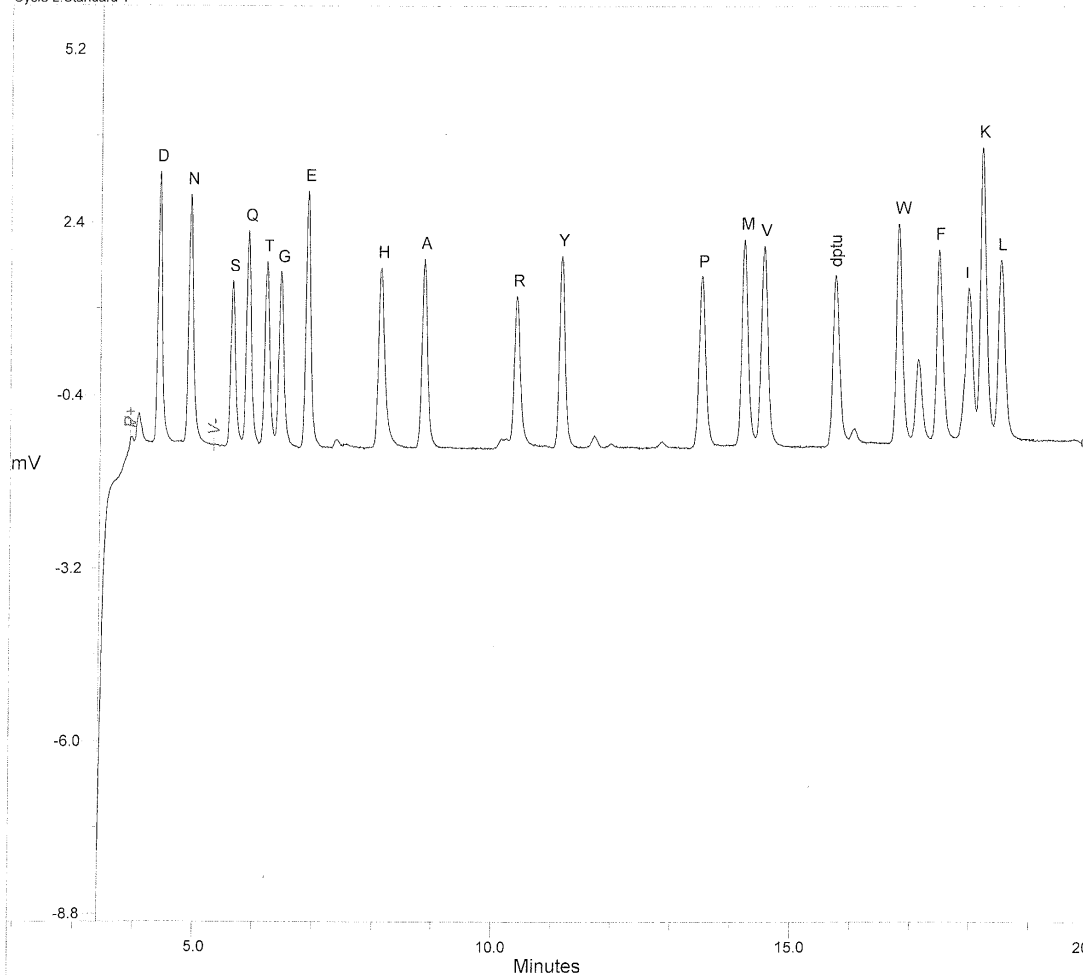
Saturday, March 31, 2012 11:25:07

120330_2A_bLG - Page 3 of 19

SequencePro™

C.D.
31 Mar 2012SGS M-Scan Ltd
SGS M-Scan job: NA
SGS M-Scan sample: NA
Operator: EPS

Cycle 2: Standard 1

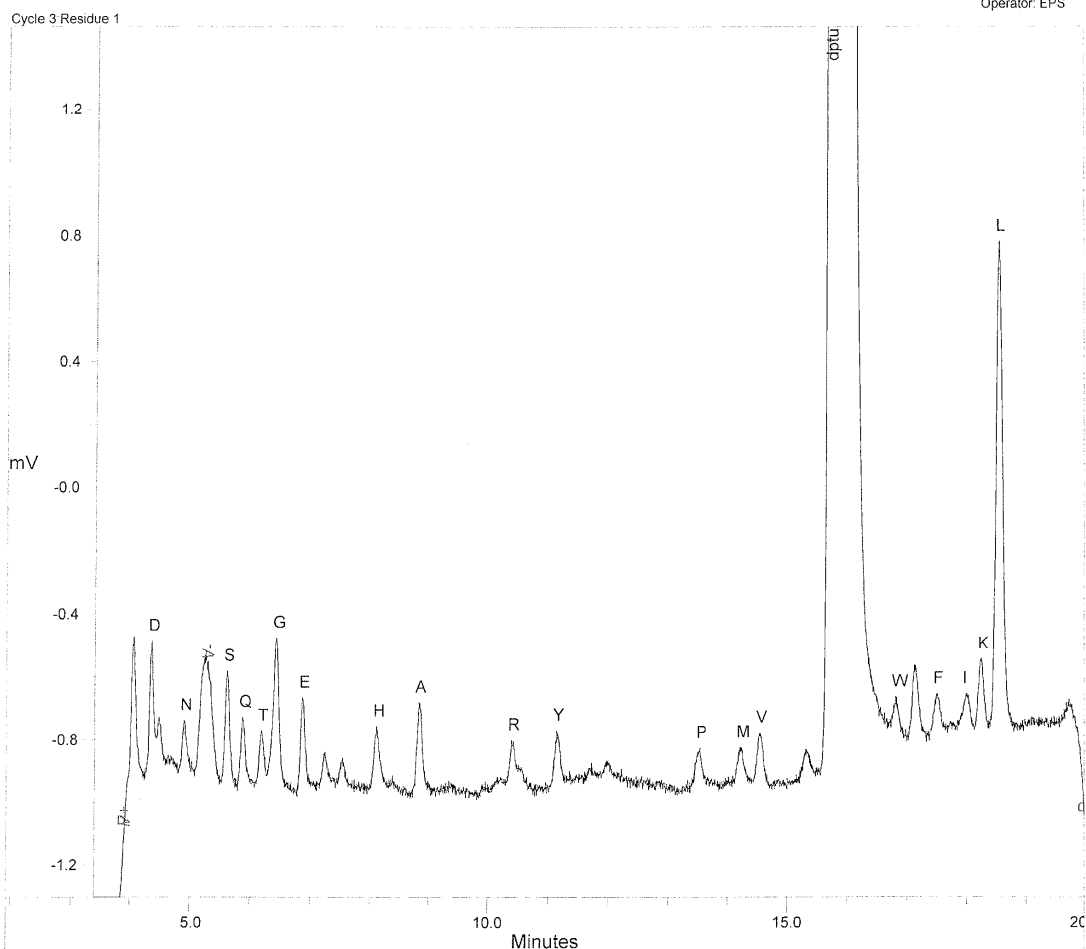


PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	3.92		0.183			12.32		0.045	
	4.03		0.466			12.41		0.045	
D	4.37	4.37	4.372	10.000		12.58		0.044	
	4.75		0.033			12.85		0.116	
N	4.90	4.90	4.021	10.000		13.25		0.025	
	5.43		0.030		P	13.52	13.52	2.748	10.000
S	5.61	5.61	2.670	10.000		13.94		0.027	
Q	5.88	5.88	3.489	10.000	M	14.23	14.23	3.346	10.000
T	6.18	6.18	2.993	10.000	V	14.56	14.56	3.245	10.000
G	6.42	6.42	2.825	10.000		15.27		0.040	
E	6.88	6.88	4.155	10.000		15.36		0.033	
	7.35		0.138			15.54		0.036	
	7.53		0.058		dptu	15.77	15.77	2.753	10.000
H	8.10	8.10	2.904	10.000		16.09		0.251	
	8.61		0.030			16.51		0.025	
A	8.84	8.84	3.053	10.000		16.61		0.023	
	9.15		0.029			16.83	16.83	3.550	10.000
	9.66		0.042			17.16		1.336	
	9.85		0.032		W	17.52	17.52	3.094	10.000
	10.14		0.167		F	18.01	18.01	2.439	10.000
	10.23		0.162		I	18.25	18.25	4.708	10.000
R	10.40	10.40	2.434	10.000	K	18.56	18.56	2.881	10.000
	10.86		0.035		L	19.07		0.051	
Y	11.15	11.15	3.111	10.000		19.14		0.039	
	11.70		0.204			19.25		0.041	
	11.98		0.078			19.60		0.066	
	12.25		0.046			19.77		0.189	

Saturday, March 31, 2012 11:25:07

120330_2A_bLG - Page 4 of 19

SequencePro™

EPS
3/17/12 2012SGS M-Scan Ltd
SGS M-Scan Job: NA
SGS M-Scan sample: NA
Operator: EPS

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.05	4.37	0.671	0.844		11.48		0.029	
	4.35		0.369			11.73		0.045	
	4.48		0.093			11.99		0.056	
	4.64		0.033			12.86		0.028	
N	4.90	4.90	0.174	0.432		12.98		0.030	
	5.25		0.415			13.15		0.023	
S	5.62	5.61	0.370	1.384		13.25		0.026	
Q	5.88	5.88	0.232	0.665		13.33		0.041	
T	6.19	6.18	0.185	0.619	P	13.53	13.52	0.130	0.474
G	6.43	6.42	0.478	1.691		13.74		0.023	
E	6.87	6.88	0.303	0.729	M	14.22	14.23	0.128	0.382
	7.12		0.025		V	14.55	14.56	0.177	0.545
	7.25		0.108			14.79		0.025	
	7.54		0.097			14.87		0.026	
	7.74		0.040			15.01		0.034	
H	8.11	8.10	0.211	0.725		15.32		0.100	
	8.39		0.049			15.51		0.026	
	8.57		0.033		dptu	15.76	15.77	10.796	39.209
A	8.83	8.84	0.292	0.956		15.98		90.285	
	9.22		0.033		W	16.81	16.83	0.120	0.337
	9.65		0.032			17.13		0.243	
	9.91		0.029		F	17.50	17.52	0.134	0.432
	10.16		0.026			17.99	18.01	0.117	0.479
R	10.40	10.40	0.142	0.585	K	18.25	18.25	0.227	0.481
	10.70		0.027		L	18.54	18.56	1.545	5.365
	10.80		0.035			19.09		0.032	
	10.84		0.032			19.22		0.031	
Y	10.96	11.15	0.035	0.607		19.34		0.031	
	11.14		0.189			19.73		0.206	
	11.35		0.048						

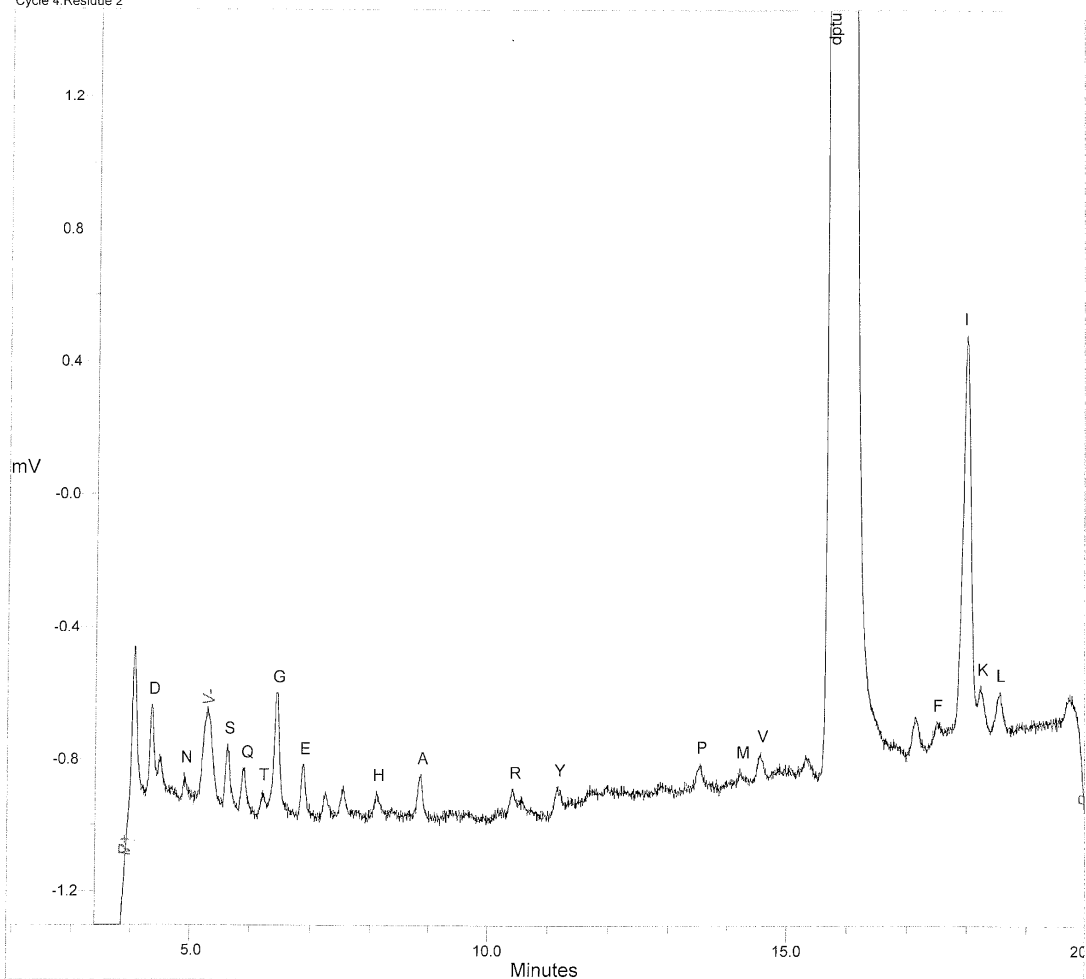
Saturday, March 31, 2012 11:25:07

120330_2A_bLG - Page 5 of 19

SequencePro™

CB
3/17/2012SGS M-Scan Ltd
SGS M-Scan job: NA
SGS M-Scan sample: NA
Operator: EPS

Cycle 4: Residue 2

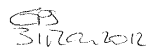


PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.04		0.613			11.98		0.049	
	4.35	4.37	0.245	0.561		12.05		0.038	
	4.48		0.080			12.23		0.034	
N	4.90	4.90	0.091	0.227		12.48		0.023	
	5.28		0.304			12.57		0.030	
S	5.62	5.61	0.203	0.759		12.64		0.029	
Q	5.90	5.88	0.141	0.405		12.86		0.034	
T	6.20	6.18	0.089	0.297		13.27		0.038	
G	6.42	6.42	0.385	1.364		13.33		0.026	
E	6.87	6.88	0.161	0.387	P	13.53	13.52	0.066	0.239
	7.04		0.033			14.06		0.032	
	7.25		0.083		M	14.21	14.23	0.051	0.153
	7.55		0.102		V	14.56	14.56	0.090	0.276
H	8.10	8.10	0.099	0.341		14.88		0.044	
	8.36		0.046			14.98		0.039	
	8.68		0.038			15.32		0.077	
A	8.86	8.84	0.147	0.483	dptu	15.77	15.77	9.369	34.027
	9.27		0.039			15.98		69.921	
	9.34		0.036			17.15		0.129	
	9.44		0.039			17.50	17.52	0.096	0.311
R	10.18	10.40	0.092	0.378	F	18.00	18.01	1.237	5.071
	10.42		0.064		I	18.23	18.25	0.142	0.302
	10.56		0.034		K	18.55	18.56	0.133	0.462
Y	11.00	11.15	0.039	0.127	L	18.76		0.033	
	11.17		0.038			19.22		0.037	
	11.33		0.035			19.34		0.027	
	11.50		0.061			19.75		0.232	
	11.69								

Saturday, March 31, 2012 11:25:07

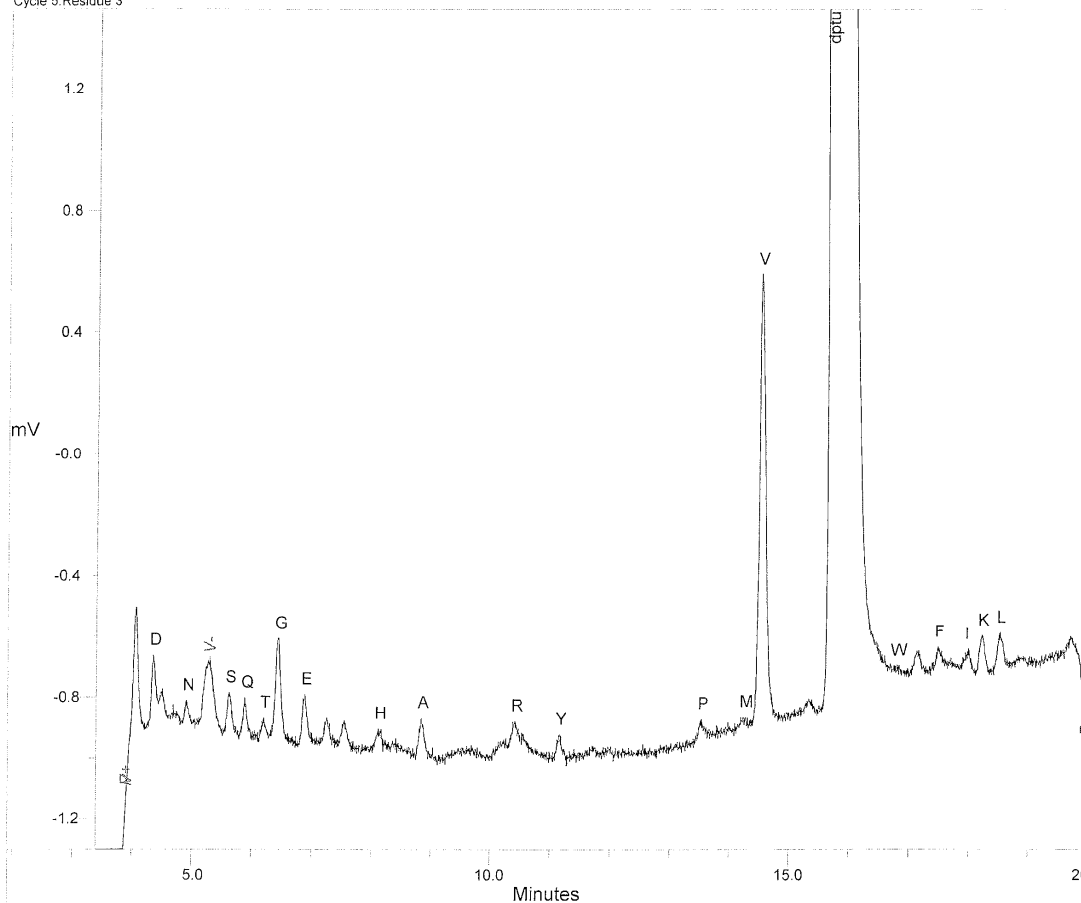
120330_2A_bLG - Page 6 of 19

SequencePro™



SGS M-Scan Ltd
 SGS M-Scan job: NA
 SGS M-Scan sample: NA
 Operator: EPS

Cycle 5: Residue 3



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.06		0.607			11.71		0.023	
	4.35	4.37	0.201	0.460		12.02		0.046	
	4.50		0.068			12.10		0.040	
N	4.90	4.90	0.080	0.200		12.32		0.027	
	5.05		0.028			12.85		0.039	
S	5.27	5.61	0.234	0.550		13.03		0.033	
Q	5.62	5.88	0.147	0.399		13.13		0.028	
	5.88		0.139			13.30		0.028	
	6.05		0.034		P	13.54	13.52	0.050	0.182
T	6.20	6.18	0.079	0.264		13.80		0.044	
G	6.44	6.42	0.341	1.208		13.88		0.027	
E	6.88	6.88	0.167	0.401	M	14.25	14.23	0.048	0.144
	7.25		0.096		V	14.56	14.56	1.478	4.553
	7.55		0.098			14.97		0.031	
	7.85		0.033			15.11		0.035	
H	8.12	8.10	0.077	0.264		15.20		0.035	
	8.20		0.038			15.35		0.055	
	8.41		0.048			15.52		0.027	
A	8.85	8.84	0.135	0.442	dptu	15.77	15.77	8.625	31.325
	9.14		0.032			15.98		61.159	
	9.30		0.039		W	16.78	16.83	0.022	0.063
	9.46		0.039			17.18		0.086	
	9.55		0.036		F	17.50	17.52	0.078	0.253
	9.62		0.035		I	18.02	18.01	0.061	0.331
	9.95		0.023		K	18.24	18.25	0.132	0.280
	10.28		0.051		L	18.55	18.56	0.124	0.431
	10.42	10.40	0.089	0.365		18.84		0.034	
Y	11.17	11.15	0.074	0.237		19.31		0.038	
	11.33		0.045			19.43		0.036	
	11.41		0.046			19.49		0.037	
	11.50		0.028			19.74		0.162	
	11.60		0.024						

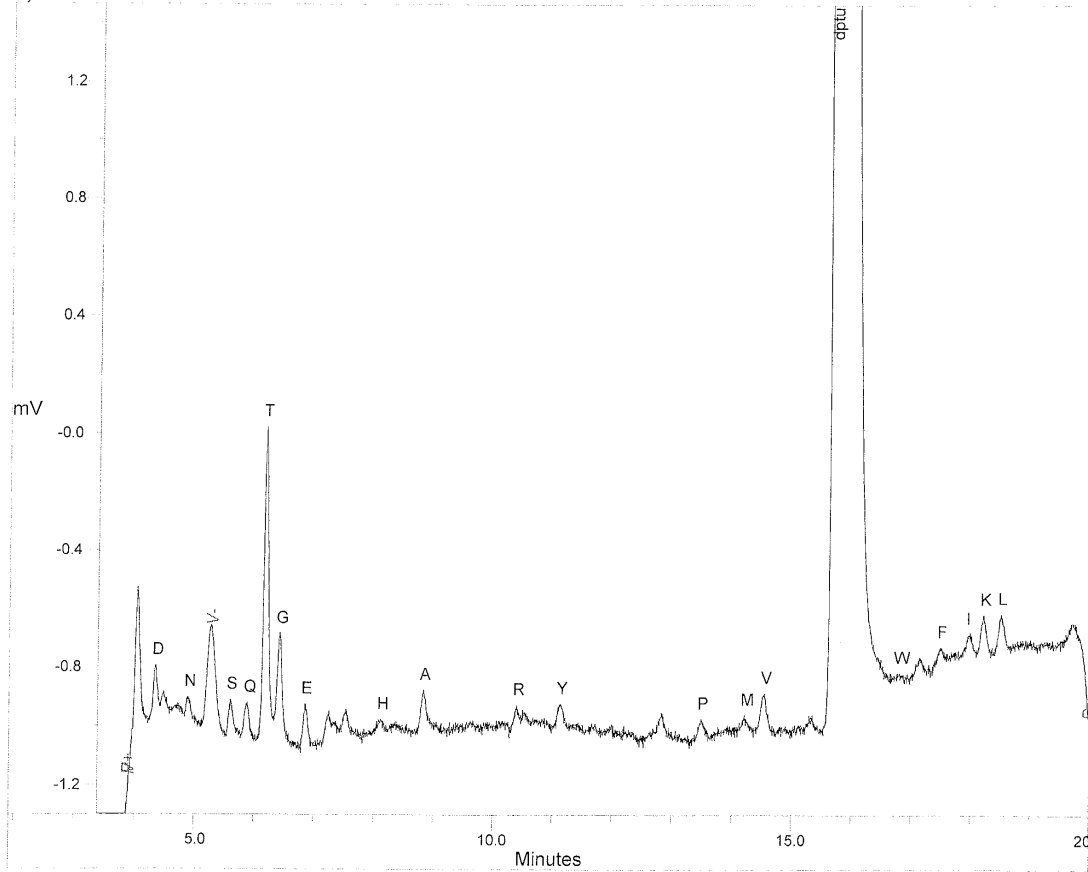
Saturday, March 31, 2012 11:25:07

120330_2A_bLG - Page 7 of 19

SequencePro™

EPS
31/03/2012SGS M-Scan Ltd
SGS M-Scan job: NA
SGS M-Scan sample: NA
Operator: EPS

Cycle 6: Residue 4

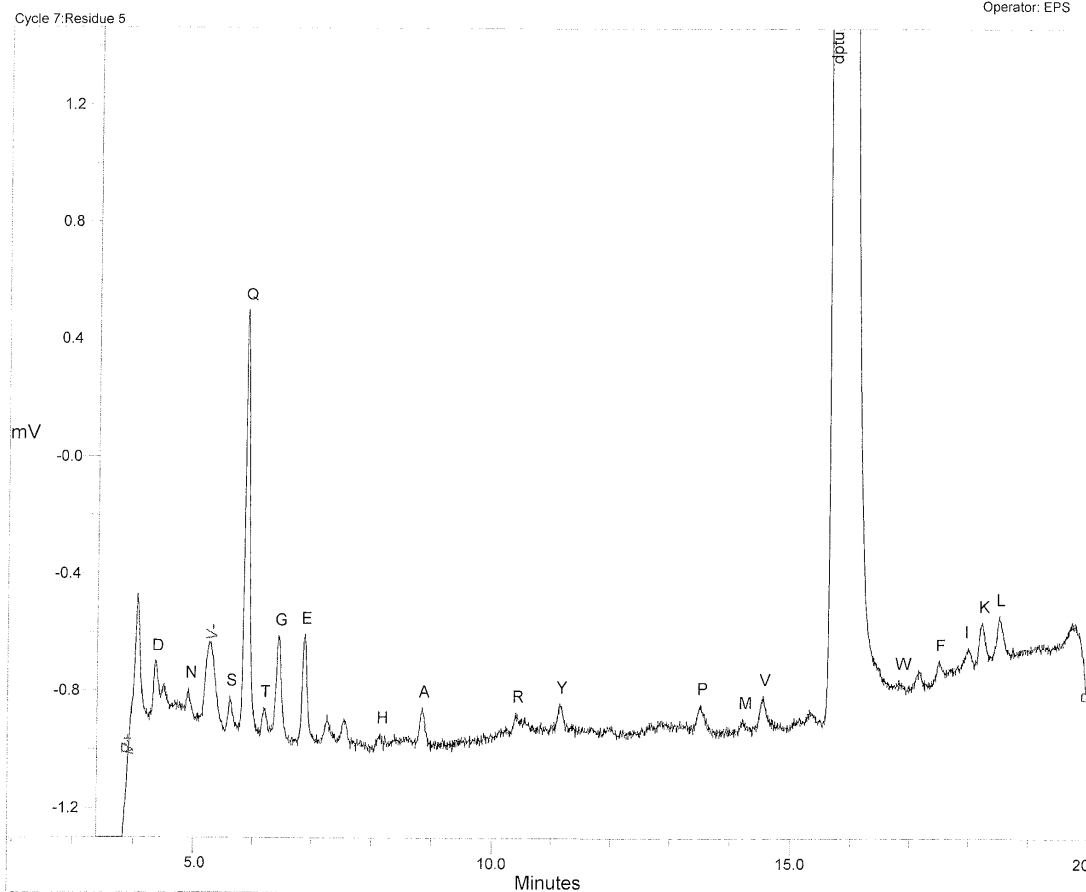


PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.03		0.660			12.00		0.046	
	4.34	4.37	0.169	0.386		12.07		0.029	
	4.48		0.062			12.21		0.046	
	4.72		0.043			12.29		0.026	
N	4.89	4.90	0.072	0.180		12.65		0.047	
	5.28		0.366			12.69		0.053	
S	5.60	5.61	0.123	0.462		12.83		0.093	
Q	5.89	5.88	0.129	0.371		13.07		0.035	
T	6.18	6.18	1.080	3.809		13.28		0.040	
G	6.42	6.42	0.382	1.353		13.50		0.087	
E	6.85	6.88	0.162	0.389	P	13.71	13.52	0.030	0.316
	7.25		0.077			13.78		0.038	
	7.54		0.088			13.92		0.023	
	7.93		0.023		M	14.22	14.23	0.075	0.225
H	8.12	8.10	0.054	0.185	V	14.56	14.56	0.147	0.452
	8.29		0.031			14.82		0.036	
	8.38		0.029			15.07		0.039	
A	8.83	8.84	0.156	0.513		15.12		0.038	
	9.05		0.039			15.35		0.055	
	9.28		0.044		dptu	15.75	15.77	9.314	33.824
	9.38		0.039			15.96		62.702	
	9.46		0.031		W	16.79	16.83	0.032	0.090
	9.61		0.039			16.95		0.033	
	9.67		0.044			17.15		0.079	
	9.74		0.036			17.30		0.044	
	9.89		0.025		F	17.50	17.52	0.067	0.216
	10.07		0.036		I	18.00	18.01	0.089	0.366
R	10.40	10.40	0.099	0.406	K	18.24	18.25	0.150	0.318
	10.51		0.072		L	18.54	18.56	0.131	0.457
	10.88		0.037			18.89		0.032	
Y	11.14	11.15	0.096	0.310		19.20		0.026	
	11.41		0.022			19.39		0.058	
	11.67		0.026			19.73		0.255	
	11.89		0.028						

Saturday, March 31, 2012 11:25:07

120330_2A_bLG - Page 8 of 19

SequencePro™

EPS
3/31/2012SGS M-Scan Ltd
SGS M-Scan job: NA
SGS M-Scan sample: NA
Operator: EPS

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.04		0.650			11.65		0.027	
	4.34	4.37	0.167	0.383		11.90		0.023	
	4.49		0.086			12.21		0.030	
	4.62		0.018			12.38		0.031	
N	4.91	4.90	0.091	0.227		12.53		0.031	
	5.26		0.291			12.69		0.032	
S	5.61	5.61	0.126	0.470		12.86		0.034	
Q	5.88	5.88	1.456	4.173		13.03		0.034	
T	6.18	6.18	0.112	0.374	P	13.52	13.52	0.058	0.212
G	6.42	6.42	0.369	1.306		13.79		0.031	
	6.66		0.029			13.96		0.038	
E	6.87	6.88	0.382	0.919		13.99		0.034	
	7.25		0.104		M	14.22	14.23	0.046	0.139
	7.53		0.101		V	14.56	14.56	0.128	0.394
H	8.14	8.10	0.061	0.211		15.07		0.029	
	8.22		0.050			15.32		0.029	
	8.38		0.038			15.49		0.023	
	8.47		0.023		dptu	15.76	15.77	8.739	31.736
	8.70		0.029			15.97		50.821	
A	8.84	8.84	0.144	0.472	W	16.82	16.83	0.035	0.097
	9.06		0.033			16.99		0.028	
	9.30		0.035			17.15		0.081	
	9.38		0.032		F	17.50	17.52	0.075	0.241
	9.63		0.040			17.61		0.020	
	9.86		0.041			17.83		0.034	
	9.98		0.032		I	18.01	18.01	0.085	0.351
	10.13		0.036		K	18.25	18.25	0.157	0.334
	10.23		0.042		L	18.53	18.56	0.163	0.567
R	10.40	10.40	0.061	0.250		18.75		0.042	
	10.55		0.039			19.11		0.025	
	10.82		0.042			19.26		0.039	
Y	11.03	11.15	0.038			19.45		0.030	
	11.15		0.100	0.321		19.75		0.050	
	11.35		0.038						

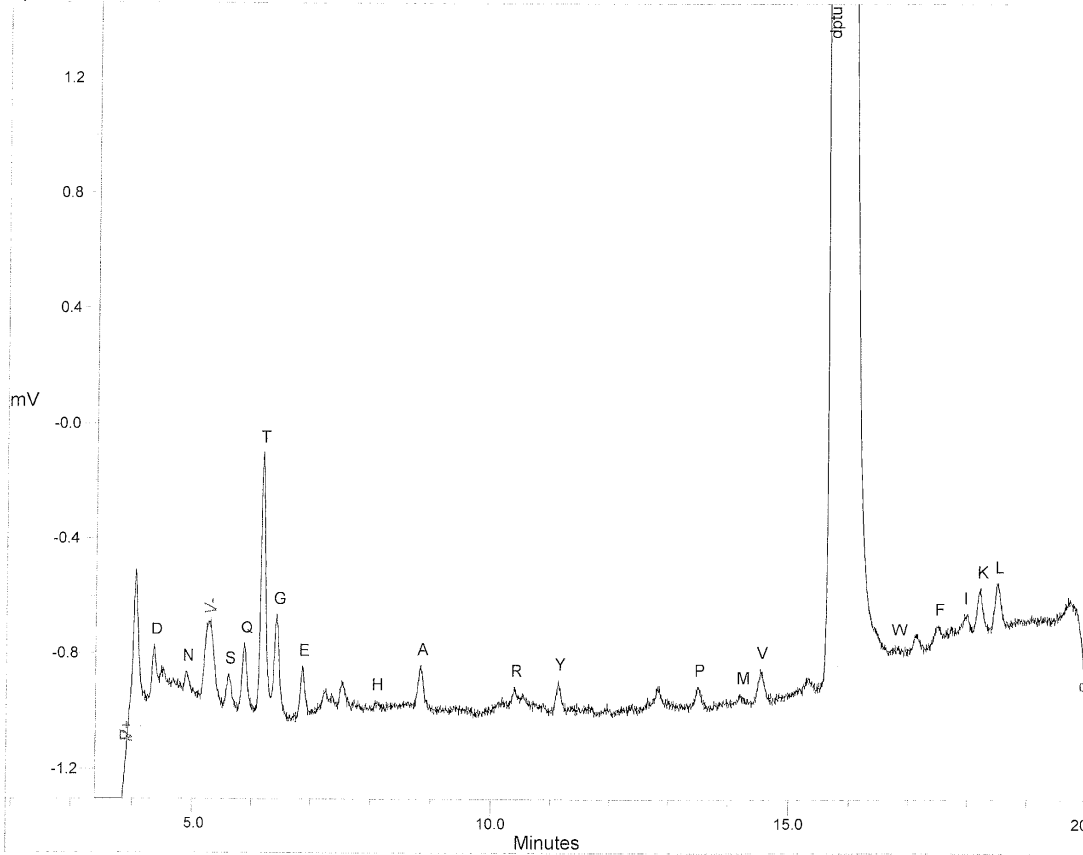
Saturday, March 31, 2012 11:25:07

120330_2A_bLG - Page 9 of 19

SequencePro™

EPS
SIRAL 2012SGS M-Scan Ltd
SGS M-Scan job: NA
SGS M-Scan sample: NA
Operator: EPS

Cycle 8, Residue 6

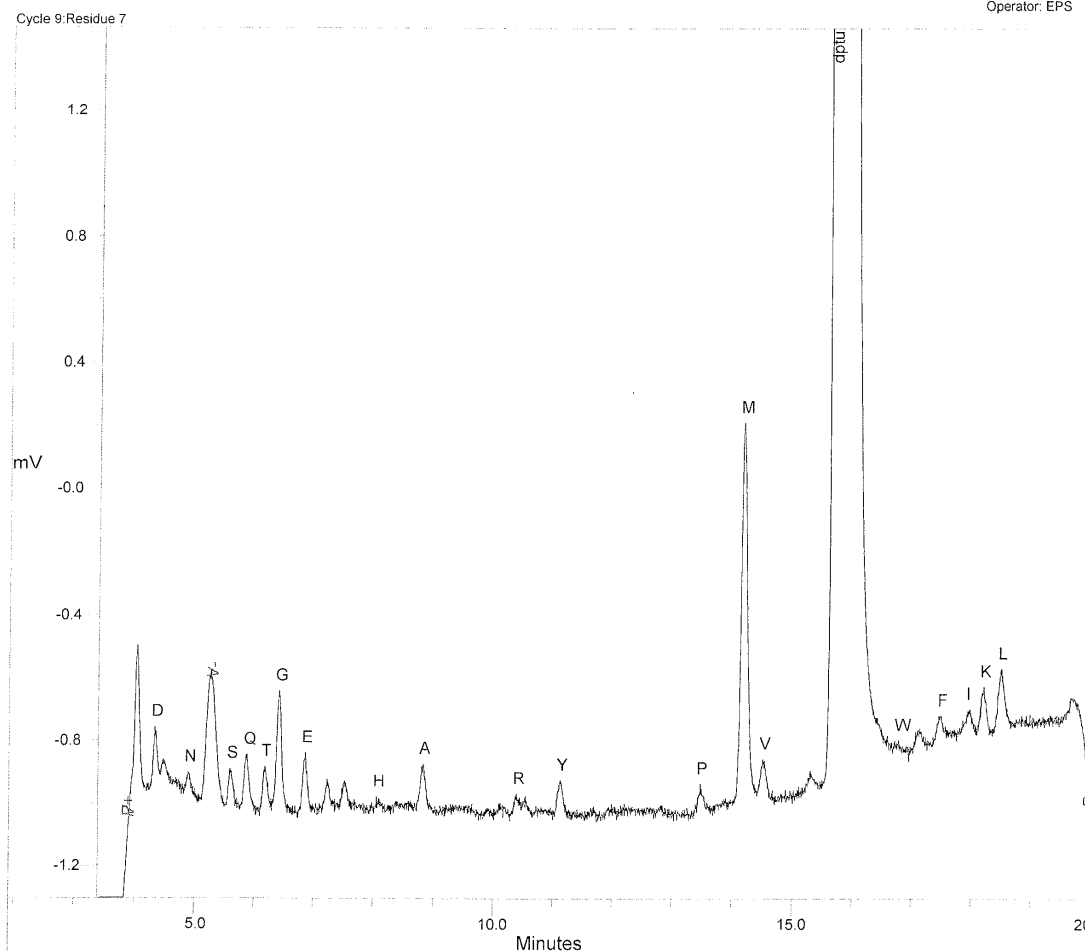


PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.04		0.643			11.45		0.044	
	4.34	4.37	0.162	0.370		11.65		0.046	
	4.47		0.060			11.80		0.019	
	4.66		0.031			12.17		0.027	
N	4.78		0.028			12.22		0.036	
	4.88	4.90	0.071	0.177		12.40		0.039	
	5.30		0.290			12.52		0.026	
	5.60	5.61	0.124	0.464		12.66		0.051	
S	5.87	5.88	0.240	0.688		12.85		0.082	
Q	6.18	6.18	0.914	3.053	P	13.50	13.52	0.073	0.265
T	6.42	6.42	0.364	1.288		13.77		0.034	
G	6.86	6.88	0.181	0.435		13.97		0.025	
E	7.26		0.081			14.20	14.23	0.036	0.107
	7.35		0.061		M	14.54	14.56	0.123	0.378
	7.52		0.097		V	14.85		0.032	
	7.72		0.031			14.96		0.022	
	7.92		0.029			15.16		0.026	
H	8.08	8.10	0.038	0.132		15.30		0.041	
	8.27		0.031		dptu	15.75	15.77	9.039	32.826
	8.41		0.026			15.96		63.034	
	8.49		0.020		W	16.79	16.83	0.035	0.099
A	8.84	8.84	0.153	0.502		17.13		0.074	
	9.16		0.027		F	17.52	17.52	0.087	0.280
	9.25		0.028			17.64		0.058	
	9.41		0.026			17.74		0.068	
	9.72		0.032			18.02	18.01	0.093	0.382
	9.90		0.029		I	18.25	18.25	0.168	0.357
	9.95		0.033		K	18.54	18.56	0.172	0.595
	10.13		0.033		L	18.74		0.032	
	10.19		0.039			18.81		0.030	
R	10.39	10.40	0.059	0.244		19.02		0.023	
	10.53		0.039			19.09		0.041	
	10.89		0.036			19.24		0.038	
Y	11.14	11.15	0.114	0.367		19.35		0.033	
	11.42		0.039			19.70		0.072	

Saturday, March 31, 2012 11:25:07

120330_2A_bLG - Page 10 of 19

SequencePro™

EPS
3/16/2012SGS M-Scan Ltd
SGS M-Scan job: NA
SGS M-Scan sample: NA
Operator: EPS

PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
	4.03		0.643			11.95		0.029	
	4.23		0.024			12.21		0.040	
D	4.33	4.37	0.179	0.410		12.35		0.027	
	4.47		0.058			12.60		0.025	
N	4.89	4.90	0.082	0.205		12.89		0.028	
	5.25		0.427			13.07		0.031	
S	5.60	5.61	0.133	0.499	P	13.48	13.52	0.095	0.347
Q	5.88	5.88	0.188	0.540		13.77		0.034	
T	6.17	6.18	0.157	0.525		13.90		0.049	
G	6.41	6.42	0.389	1.378	M	14.20	14.23	1.231	3.678
E	6.66	6.88	0.199	0.479	V	14.53	14.56	0.151	0.466
	7.23		0.109			14.82		0.031	
	7.52		0.098			14.90		0.039	
	7.70		0.028			15.10		0.036	
	7.98		0.035			15.32		0.074	
H	8.06	8.10	0.043	0.149	dptu	15.74	15.77	9.069	32.937
	8.19		0.028			15.95		65.519	
	8.39		0.039			16.73		0.039	
	8.58		0.032			16.80	16.83	0.040	0.112
A	8.82	8.84	0.148	0.484	W	17.15		0.054	
	9.33		0.029		F	17.51	17.52	0.093	0.301
	9.60		0.027			17.73		0.043	
	9.90		0.036		I	17.99	18.01	0.097	0.398
	10.13		0.057		K	18.23	18.25	0.160	0.340
R	10.39	10.40	0.085	0.347	L	18.52	18.56	0.201	0.697
	10.54		0.073			18.86		0.029	
	10.73		0.036			19.30		0.029	
Y	11.13	11.15	0.123	0.396		19.44		0.033	
	11.37		0.025			19.48		0.033	
	11.70		0.048			19.68		0.059	

Saturday, March 31, 2012 11:25:07

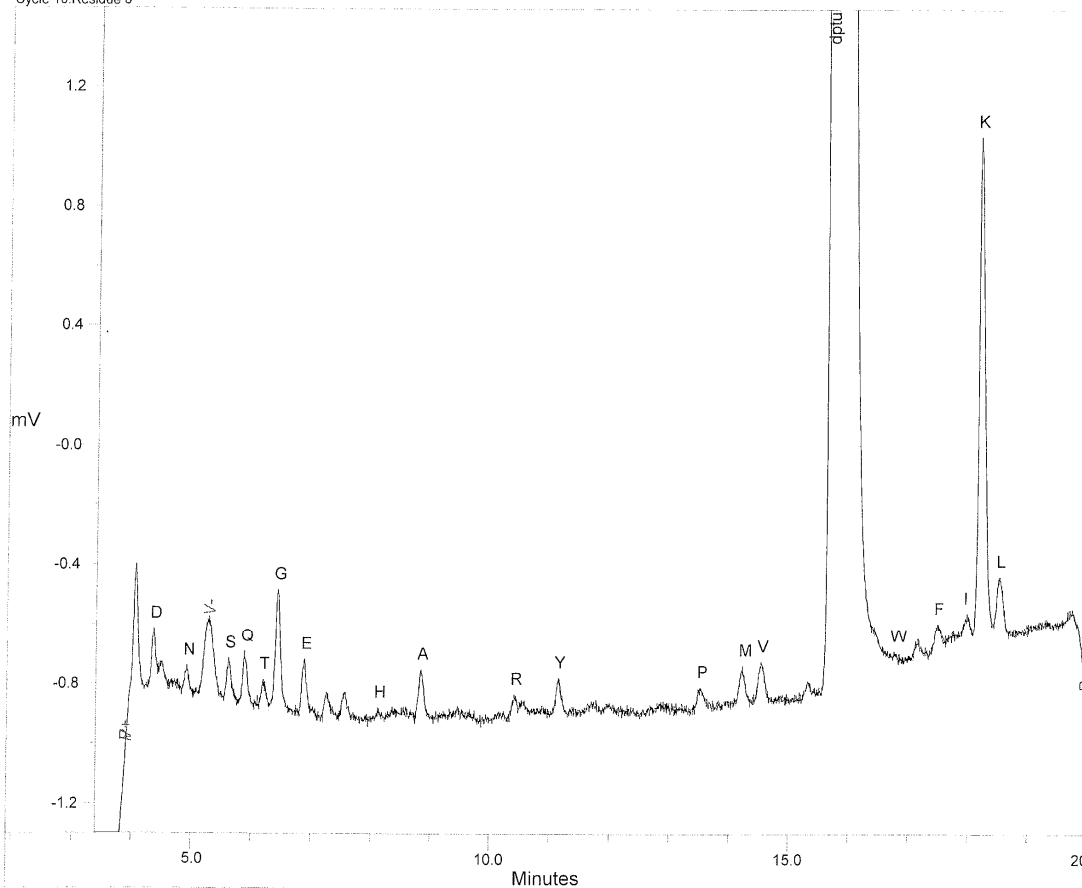
120330_2A_bLG - Page 11 of 19

SequencePro™

EP
3/16/2012

SGS M-Scan Ltd
SGS M-Scan job: NA
SGS M-Scan sample: NA
Operator: EPS

Cycle 10, Residue 8



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.06		0.603			11.95		0.018	
	4.36	4.37	0.176	0.403		12.20		0.039	
	4.47		0.054			12.30		0.045	
	4.66		0.031			12.48		0.031	
N	4.92	4.90	0.103	0.256		12.81		0.031	
	5.29		0.286			13.05		0.040	
S	5.62	5.61	0.157	0.587		13.21		0.040	
Q	5.88	5.88	0.190	0.543		13.30		0.035	
T	6.20	6.18	0.107	0.359	P	13.53	13.52	0.075	0.274
G	6.43	6.42	0.416	1.474		13.83		0.036	
E	6.89	6.88	0.187	0.451		13.95		0.016	
	7.12		0.032			14.10		0.037	
	7.25		0.095		M	14.23	14.23	0.139	0.415
	7.56		0.096		V	14.55	14.56	0.144	0.444
	7.84		0.025			14.91		0.035	
	7.93		0.041			15.34		0.062	
	8.03		0.027		dptu	15.77	15.77	8.306	30.165
H	8.11	8.10	0.045	0.157		15.98		58.696	
	8.28		0.033			16.73		0.020	
	8.36		0.046		W	16.79	16.83	0.034	0.097
	8.59		0.037			16.96		0.023	
	8.68		0.036			17.19		0.066	
A	8.85	8.84	0.175	0.574	F	17.51	17.52	0.087	0.282
	9.10		0.042			17.77		0.053	
	9.22		0.037		I	18.00	18.01	0.105	0.429
	9.32		0.042		K	18.24	18.25	1.896	3.602
	9.46		0.046		L	18.55	18.56	0.212	0.736
	9.94		0.035			18.80		0.032	
	10.25		0.032			19.00		0.042	
R	10.42	10.40	0.075	0.310		19.34		0.032	
Y	11.16	11.15	0.132	0.425		19.52		0.062	
	11.49		0.024			19.75		0.204	
	11.65		0.031						

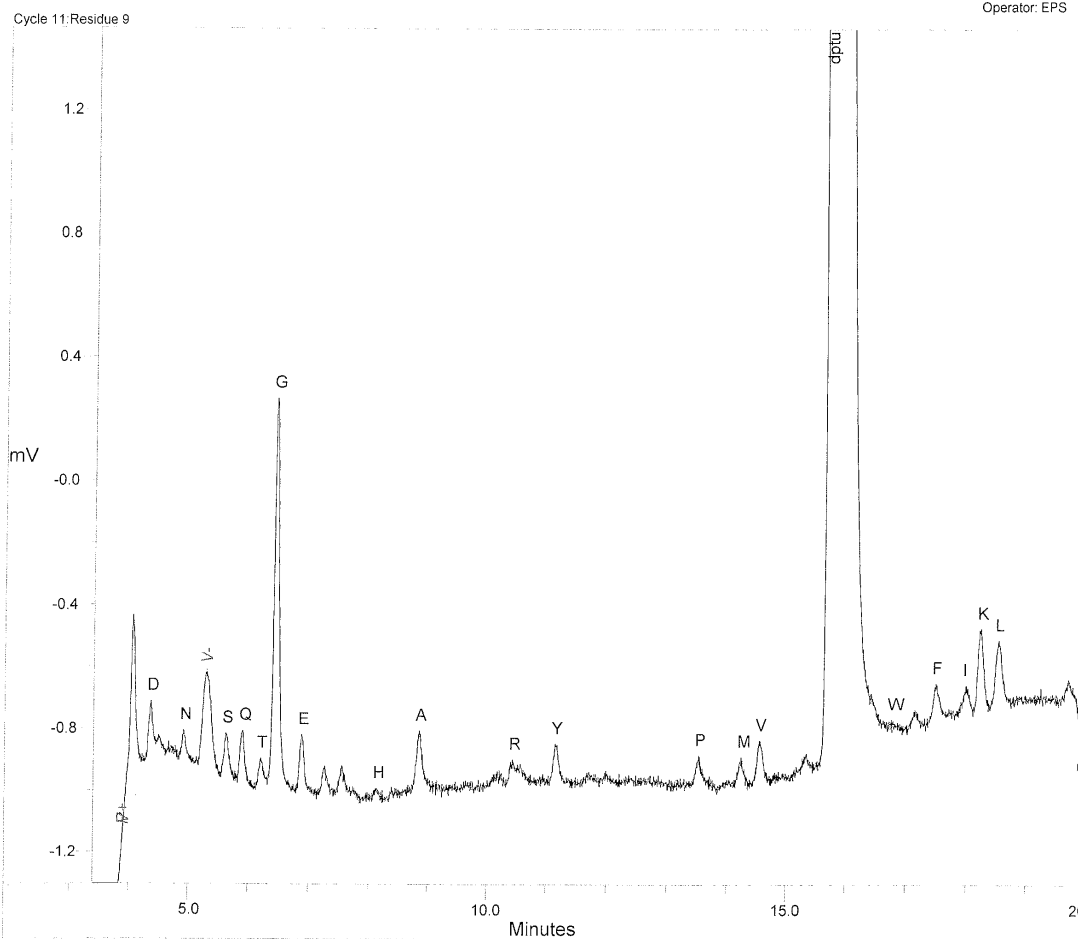
Saturday, March 31, 2012 11:25:07

120330_2A_bLG - Page 12 of 19

SequencePro™



SGS M-Scan Ltd
 SGS M-Scan job: NA
 SGS M-Scan sample: NA
 Operator: EPS



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.05		0.666			12.38		0.023	
	4.36	4.37	0.169	0.387		12.57		0.026	
	4.60		0.046			13.04		0.036	
	4.66		0.041			13.23		0.041	
N	4.91	4.90	0.100	0.250		13.29		0.039	
	5.30		0.312		P	13.53	13.52	0.092	0.336
	5.63	5.61	0.143	0.537		13.70		0.030	
	5.90	5.88	0.177	0.506		14.04		0.026	
S	6.20	6.18	0.104	0.348	M	14.24	14.23	0.093	0.278
	6.44	6.42	1.274	4.511	V	14.55	14.56	0.149	0.458
E	6.75		0.029			14.82		0.036	
	6.88	6.88	0.185	0.446		14.88		0.034	
	7.27		0.058			15.18		0.025	
	7.40		0.026			15.35		0.064	
H	7.55		0.094			15.54		0.032	
	8.14	8.10	0.047	0.162	dptu	15.78	15.77	7.736	28.095
	8.39		0.043			15.98		59.439	
	8.45		0.036		W	16.78	16.83	0.039	0.109
A	8.62		0.028			16.92		0.023	
	8.67		0.038			17.17		0.065	
	8.85	8.84	0.208	0.683	F	17.51	17.52	0.128	0.413
	9.25		0.045		I	18.02	18.01	0.103	0.422
R	9.40		0.027		K	18.26	18.25	0.277	0.588
	10.02		0.035		L	18.55	18.56	0.215	0.747
	10.22		0.058			18.80		0.034	
	10.44	10.40	0.061	0.251		19.00		0.029	
Y	10.95		0.036			19.20		0.032	
	11.16	11.15	0.132	0.424		19.27		0.055	
	11.48		0.025			19.36		0.031	
	11.68		0.028			19.53		0.046	
	11.99		0.049			19.74		0.203	

Saturday, March 31, 2012 11:25:07

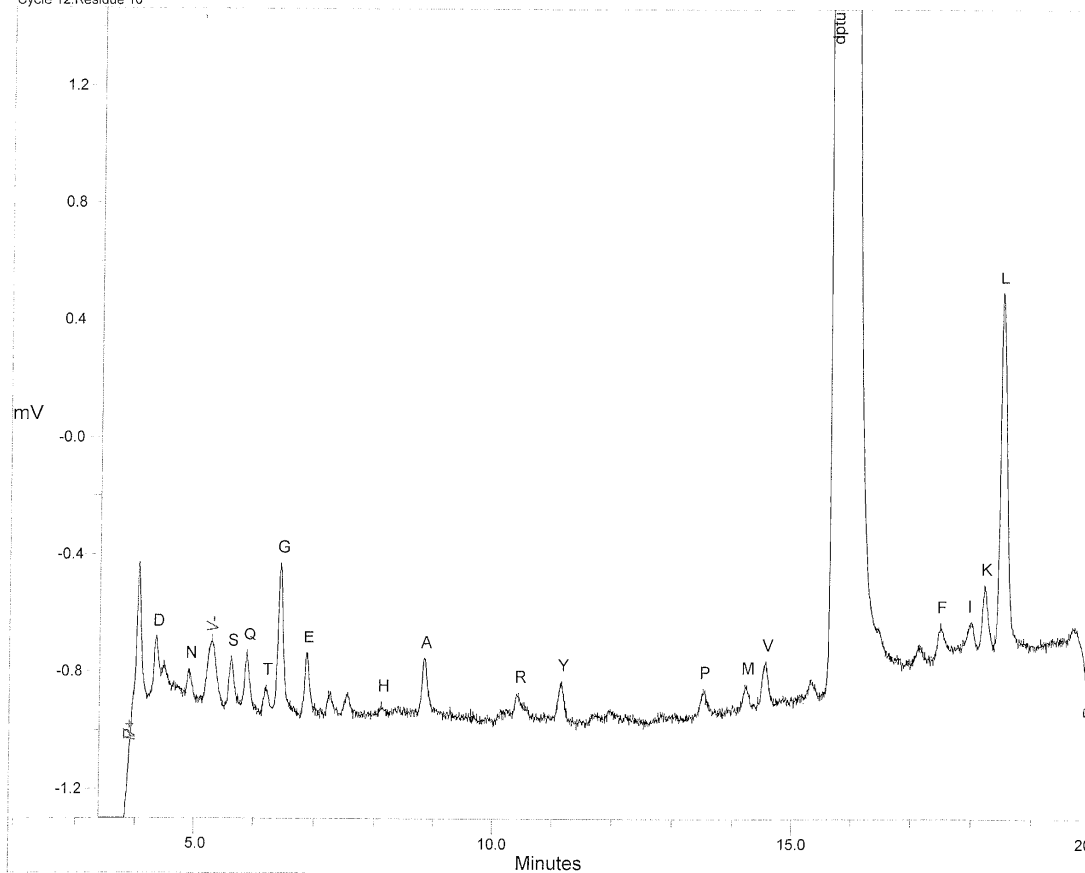
120330_2A_bLG - Page 13 of 19

SequencePro™

3/31/2012

SGS M-Scan Ltd
SGS M-Scan job: NA
SGS M-Scan sample: NA
Operator: EPS

Cycle 12: Residue 10



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.05		0.652			11.47		0.028	
	4.35	4.37	0.172	0.394		11.68		0.024	
	4.48		0.070			12.22		0.037	
	4.65		0.034			12.35		0.038	
N	4.90	4.90	0.106	0.264		12.72		0.024	
	5.04		0.019			13.05		0.039	
	5.28		0.222			13.24		0.029	
S	5.62	5.61	0.170	0.639	P	13.51	13.52	0.093	0.338
Q	5.88	5.88	0.198	0.567		13.70		0.028	
T	6.20	6.18	0.104	0.349		13.91		0.038	
G	6.43	6.42	0.519	1.839		14.00		0.039	
E	6.87	6.88	0.217	0.522	M	14.22	14.23	0.099	0.296
	7.10		0.032			14.35		0.028	
	7.23		0.086		V	14.57	14.56	0.162	0.499
	7.55		0.088			14.86		0.028	
	7.83		0.030			15.18		0.041	
	7.90		0.028			15.34		0.081	
	8.02		0.025		dptu	15.77	15.77	8.217	29.841
H	8.11	8.10	0.042	0.146		15.97		63.833	
	8.34		0.025			16.98		0.035	
	8.48		0.030			17.15		0.062	
	8.68		0.028			17.31		0.033	
A	8.84	8.84	0.191	0.626	F	17.50	17.52	0.142	0.460
	9.43		0.030			17.68		0.056	
	9.63		0.046			17.78		0.054	
	9.83		0.046		I	18.01	18.01	0.126	0.517
	9.92		0.029		K	18.23	18.25	0.241	0.512
	10.09		0.027		L	18.53	18.56	1.226	4.255
	10.23		0.036			19.15		0.045	
R	10.44	10.40	0.077	0.315		19.23		0.038	
	10.74		0.033			19.37		0.039	
	10.82		0.031			19.45		0.036	
Y	10.87		0.038			19.71		0.149	
	11.16	11.15	0.142	0.456					

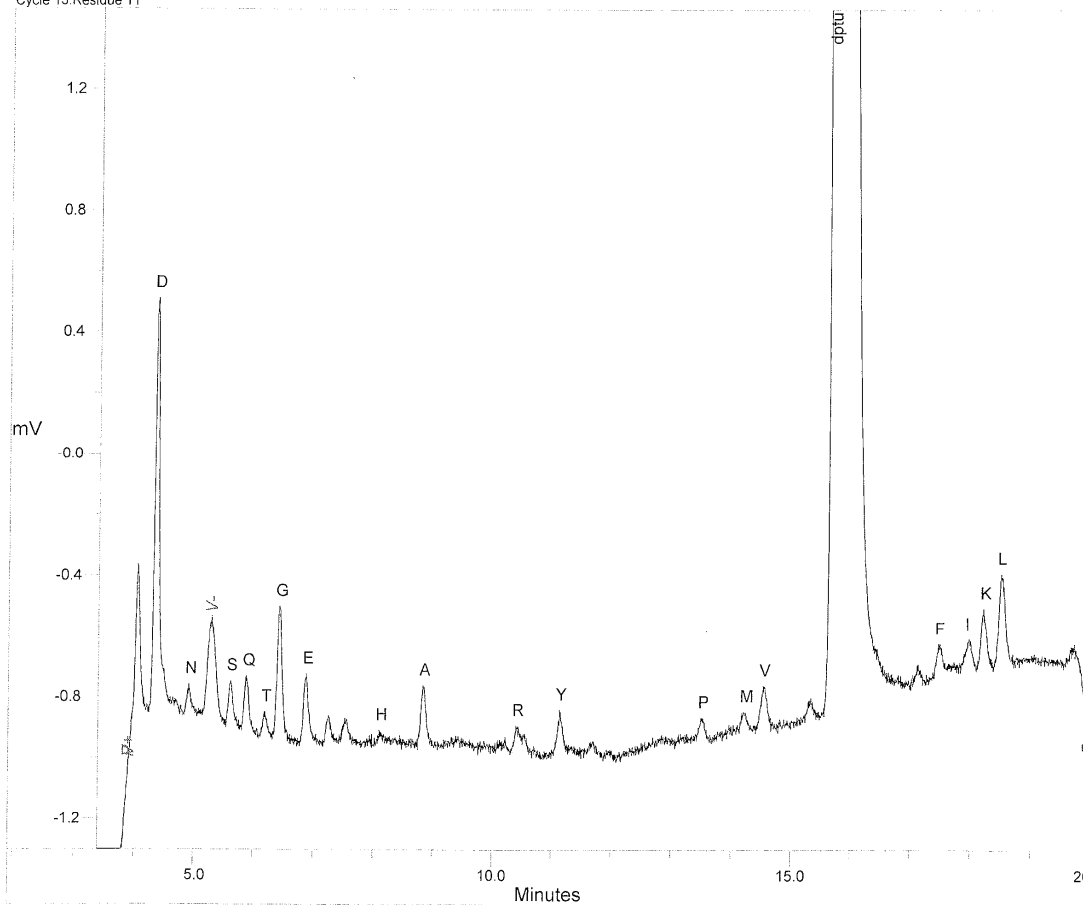
Saturday, March 31, 2012 11:25:07

120330_2A_bLG - Page 14 of 19

SequencePro™

EPS
3/11/2012SGS M-Scan Ltd
SGS M-Scan job: NA
SGS M-Scan sample: NA
Operator: EPS

Cycle 13: Residue 11



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.04		0.650			11.70		0.047	
	4.35		1.356			11.93		0.038	
	4.67	4.37	0.026	3.101		12.15		0.034	
	4.78		0.032			12.31		0.030	
N	4.91	4.90	0.101	0.252		12.51		0.033	
	5.30		0.335			12.70		0.037	
	5.61	5.61	0.157	0.589		12.85		0.036	
S	5.88	5.88	0.192	0.551		13.03		0.032	
Q	6.19	6.18	0.093	0.312		13.16		0.039	
T	6.42	6.42	0.450	1.592	P	13.51	13.52	0.082	0.298
G	6.88	6.88	0.231	0.555		13.84		0.033	
E	7.07		0.021			13.97		0.040	
	7.25		0.095		M	14.22	14.23	0.072	0.216
	7.53		0.086			14.41		0.027	
	7.85		0.023		V	14.54	14.56	0.152	0.468
	7.98		0.045			14.82		0.041	
	8.10	8.10	0.052	0.181		14.89		0.029	
	8.37		0.030			15.12		0.031	
H	8.45		0.043			15.31		0.029	
	8.65		0.030			15.48		0.027	
	8.84	8.84	0.202	0.663	dptu	15.76	15.77	8.177	29.697
	9.19		0.029			15.97		65.500	
	9.32		0.036			16.95		0.029	
A	9.39		0.039			17.15		0.078	
	9.80		0.035		F	17.49	17.52	0.101	0.328
	10.13		0.039			17.75		0.033	
	10.41	10.40	0.079	0.323		18.00	18.01	0.111	0.453
	10.71		0.038		I	18.24	18.25	0.210	0.446
R	10.89		0.026		K	18.52	18.56	0.311	1.079
	11.14	11.15	0.156	0.500	L	19.02		0.022	
	11.30		0.038			19.20		0.031	
Y	11.53		0.032			19.76		0.050	

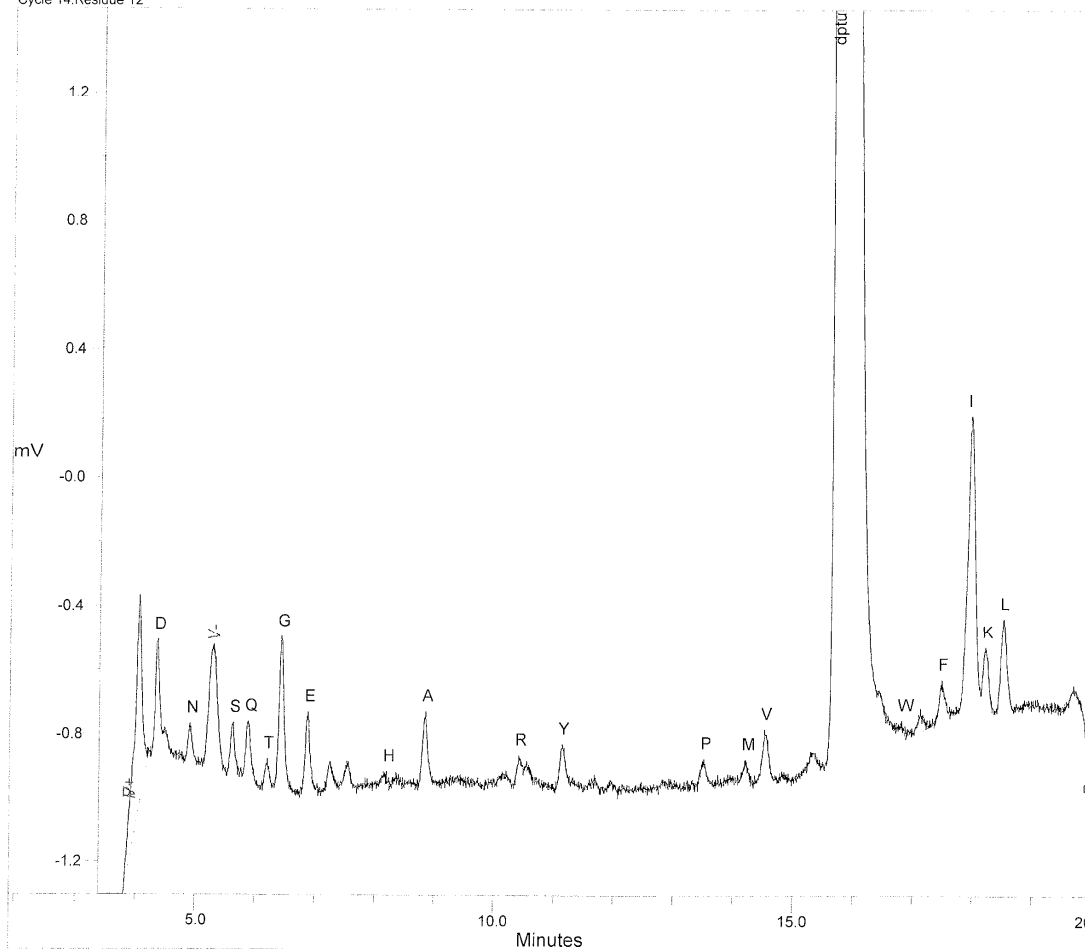
Saturday, March 31, 2012 11:25:07

120330_2A_bLG - Page 15 of 19

SequencePro™

CIS
3/17/2012SGS M-Scan Ltd
SGS M-Scan job: NA
SGS M-Scan sample: NA
Operator: EPS

Cycle 14:Residue 12



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.04		0.676		Y	11.15	11.15	0.145	0.465
	4.35	4.37	0.333	0.761		11.69		0.061	
	4.73		0.033			11.96		0.056	
N	4.91	4.90	0.130	0.323		12.39		0.025	
	5.29		0.393			12.48		0.034	
S	5.63	5.61	0.176	0.661		12.82		0.039	
Q	5.89	5.88	0.199	0.572		13.17		0.017	
T	6.20	6.18	0.103	0.345		13.34		0.037	
G	6.42	6.42	0.490	1.736	P	13.53	13.52	0.099	0.360
E	6.88	6.88	0.266	0.641		13.93		0.036	
	7.26		0.102			13.96		0.034	
	7.53		0.091		M	14.22	14.23	0.085	0.255
	7.77		0.031		V	14.54	14.56	0.170	0.524
	7.84		0.047			14.83		0.032	
	7.95		0.035			15.30		0.059	
	8.01		0.035			15.50		0.028	
H	8.20	8.10	0.063	0.216	dptu	15.76	15.77	8.249	29.959
	8.60		0.029			15.97		67.914	
	8.65		0.034		W	16.82	16.83	0.045	0.126
A	8.85	8.84	0.242	0.794		17.15		0.073	
	9.20		0.033		F	17.50	17.52	0.124	0.399
	9.28		0.031			17.66		0.032	
	9.57		0.035		I	18.00	18.01	0.939	3.850
	9.74		0.034		K	18.23	18.25	0.212	0.451
	9.87		0.032		L	18.54	18.56	0.296	1.027
	10.00		0.029			18.89		0.034	
R	10.14	10.40	0.035	0.421		19.25		0.032	
	10.42		0.102			19.40		0.060	
	10.55		0.079			19.72		0.253	

Saturday, March 31, 2012 11:25:07

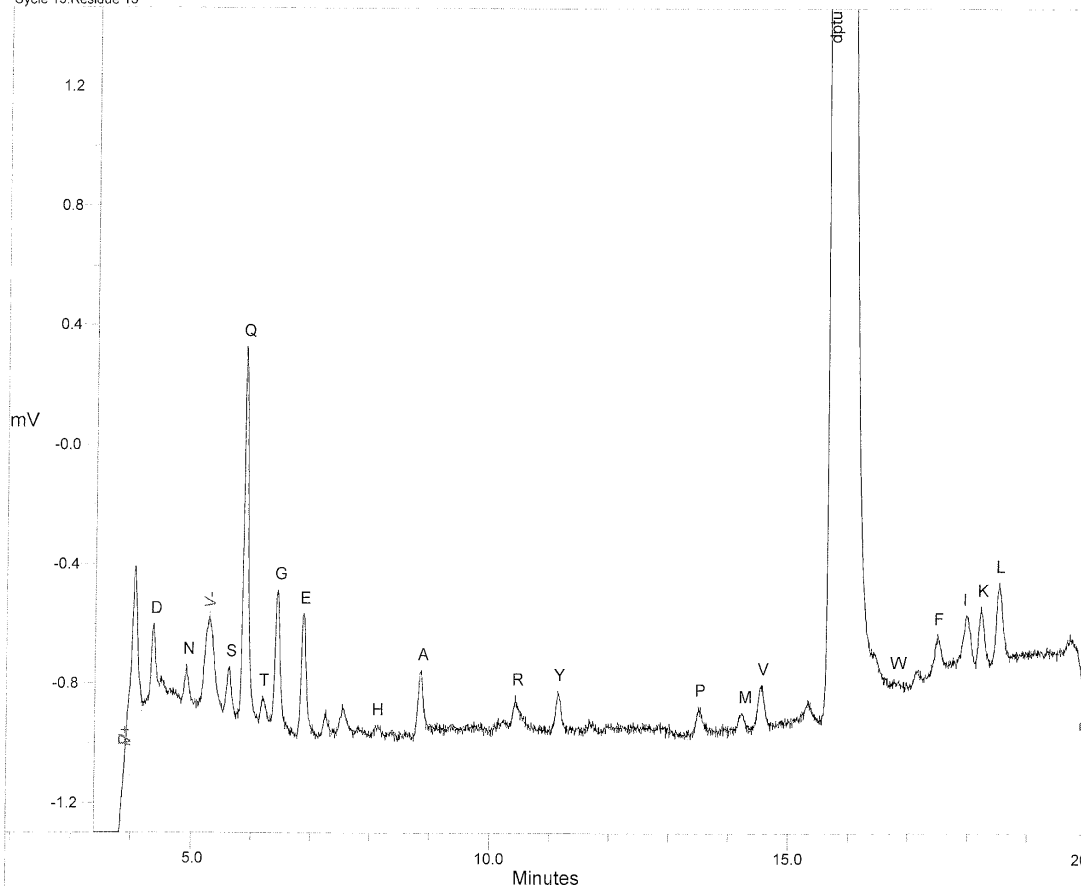
120330_2A_bLG - Page 16 of 19

SequencePro™

ED
31 Mar 2012

SGS M-Scan Ltd
SGS M-Scan job: NA
SGS M-Scan sample: NA
Operator: EPS

Cycle 15: Residue 13



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.05		0.625			11.67		0.058	
	4.35		0.241			11.99		0.026	
	4.48	4.37	0.043	0.551		12.15		0.033	
	4.68		0.026			12.41		0.036	
N	4.91	4.90	0.138	0.344		12.49		0.027	
	5.28		0.320			12.57		0.030	
S	5.63	5.61	0.169	0.635		12.87		0.037	
Q	5.88	5.88	1.249	3.580		13.02		0.051	
T	6.19	6.18	0.098	0.328		13.34		0.033	
G	6.44	6.42	0.475	1.681	P	13.50	13.52	0.100	0.364
E	6.87	6.88	0.414	0.998		13.91		0.046	
	7.26		0.067			14.00		0.040	
	7.38		0.030		M	14.24	14.23	0.070	0.208
	7.54		0.113		V	14.56	14.56	0.160	0.493
	7.80		0.032			15.15		0.037	
H	8.10	8.10	0.025	0.087		15.33		0.088	
	8.38		0.038		dptu	15.76	15.77	7.711	28.004
	8.49		0.032			15.97		55.030	
	8.58		0.029		W	16.78	16.83	0.033	0.094
A	8.86	8.84	0.220	0.721		16.85		0.029	
	9.13		0.037			16.94		0.035	
	9.52		0.026			17.18		0.053	
	9.64		0.038		F	17.50	17.52	0.127	0.410
	9.74		0.047			17.77		0.038	
	9.85		0.044		I	17.99	18.01	0.181	0.741
	10.15		0.052		K	18.24	18.25	0.211	0.448
	10.24		0.040		L	18.54	18.56	0.278	0.967
R	10.43	10.40	0.095	0.391		18.92		0.033	
	10.84		0.030			19.04		0.031	
Y	11.14	11.15	0.145	0.465		19.13		0.030	
	11.35		0.029			19.35		0.061	
	11.48		0.030			19.40		0.077	
	11.60		0.039			19.73		0.259	

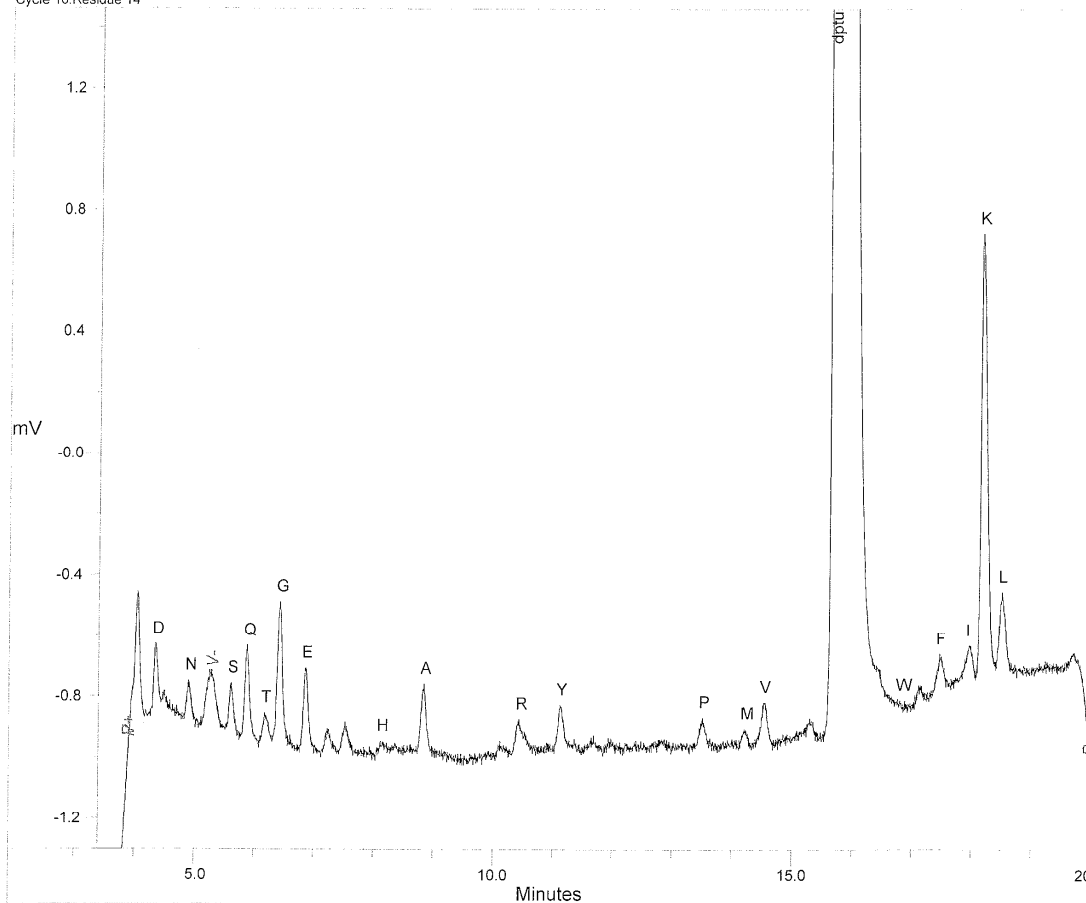
Saturday, March 31, 2012 11:25:07

120330_2A_bLG - Page 17 of 19

SequencePro™

CD
3/11/2012SGS M-Scan Ltd
SGS M-Scan job: NA
SGS M-Scan sample: NA
Operator: EPS

Cycle 16/Residue 14



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.04		0.578			12.41		0.036	
N	4.34	4.37	0.219	0.500		12.73		0.036	
	4.90	4.90	0.144	0.357		12.83		0.021	
	5.04		0.031			13.03		0.033	
	5.28		0.207			13.32		0.026	
S	5.61	5.61	0.185	0.694		13.38		0.028	
Q	5.88	5.88	0.325	0.932	P	13.52	13.52	0.105	0.383
T	6.17	6.18	0.114	0.383		13.87		0.027	
G	6.42	6.42	0.477	1.689		14.01		0.030	
E	6.86	6.88	0.279	0.672	M	14.21	14.23	0.069	0.206
	7.23		0.081		V	14.53	14.56	0.156	0.480
	7.53		0.108			14.78		0.034	
	7.84		0.031			14.86		0.039	
H	8.12	8.10	0.053	0.182		15.10		0.035	
	8.36		0.036			15.31		0.035	
	8.49		0.027			15.49		0.030	
	8.51		0.032		dptu	15.75	15.77	7.791	28.294
A	8.95	8.84	0.234	0.767		15.96		58.608	
	9.20		0.033		W	16.82	16.83	0.026	0.074
	9.62		0.037			16.99		0.027	
	9.70		0.038			17.14		0.065	
	9.78		0.039		F	17.48	17.52	0.126	0.407
	9.93		0.033			17.75		0.038	
	10.18		0.027		I	17.98	18.01	0.127	0.520
R	10.44	10.40	0.102	0.418	K	18.23	18.25	1.467	3.116
	10.90		0.032		L	18.53	18.56	0.271	0.941
Y	11.13	11.15	0.150	0.482		18.82		0.022	
	11.46		0.030			19.02		0.031	
	11.55		0.024			19.25		0.037	
	11.95		0.057			19.39		0.033	
	12.13		0.039			19.75		0.041	
	12.23		0.038						

Saturday, March 31, 2012 11:25:07

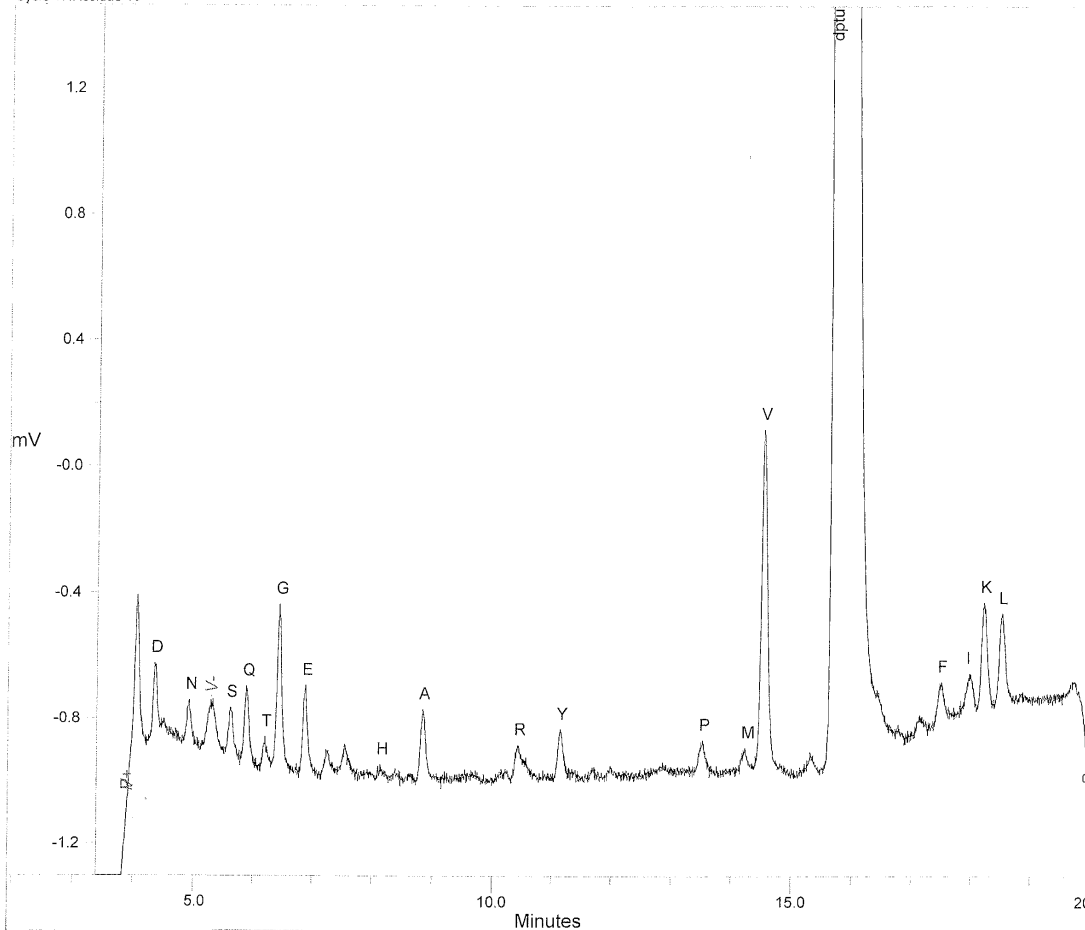
120330_2A_bLG - Page 18 of 19

SequencePro™

EB
31/03/2012

SGS M-Scan Ltd
SGS M-Scan job: NA
SGS M-Scan sample: NA
Operator: EPS

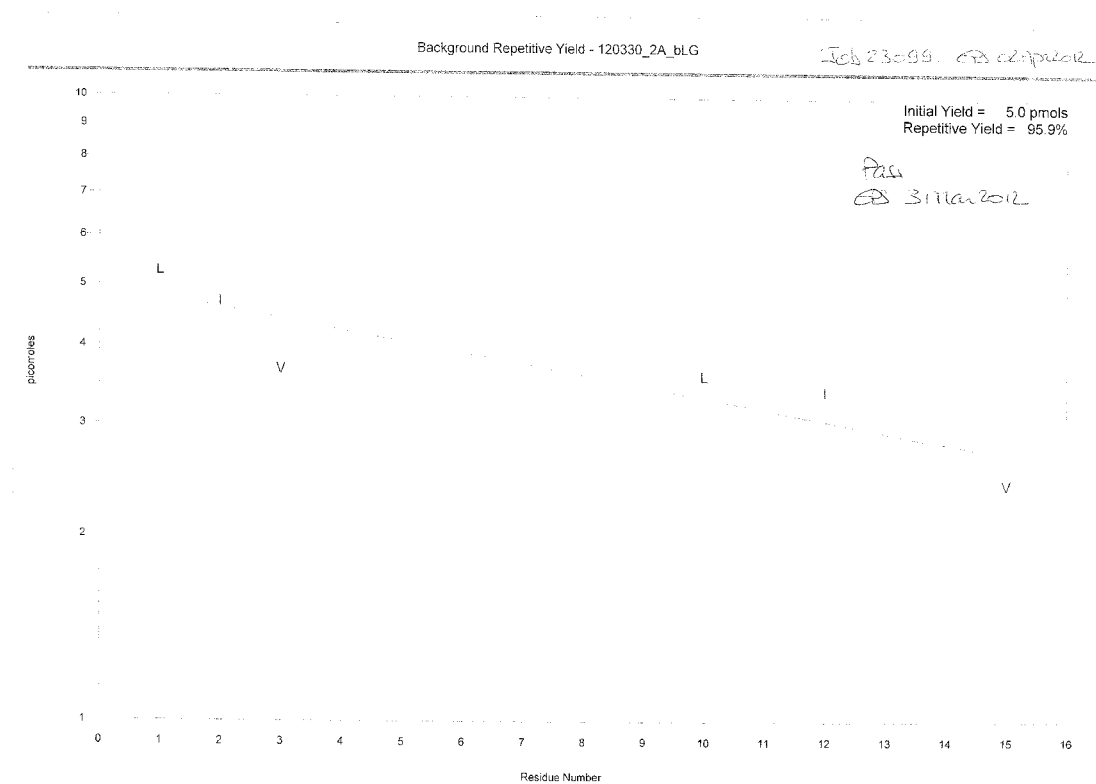
Cycle 17: Residue 15



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.05		0.666			11.99		0.039	
	4.34	4.37	0.235	0.537		12.12		0.024	
	4.50		0.042			12.25		0.034	
	4.69		0.040			12.49		0.034	
N	4.92	4.90	0.150	0.374		12.62		0.032	
	5.32		0.180			12.86		0.033	
	5.61	5.61	0.174	0.651	P	13.53	13.52	0.119	0.432
Q	5.88	5.88	0.256	0.733		13.93		0.021	
T	6.20	6.18	0.113	0.379		14.05		0.029	
G	6.43	6.42	0.540	1.910	M	14.23	14.23	0.086	0.258
E	6.88	6.88	0.291	0.701	V	14.55	14.56	1.088	3.353
	7.22		0.083			15.01		0.032	
	7.54		0.104			15.15		0.033	
	7.93		0.030			15.23		0.043	
H	8.13	8.10	0.041	0.143		15.32		0.077	
	8.38		0.053			15.76		8.021	
	8.62		0.039		dptu	15.97	15.77	62.657	29.130
	8.84	8.84	0.233	0.764		16.95		0.038	
A	9.21		0.038			17.15		0.051	
	9.38		0.030			17.51	17.52	0.132	0.428
	9.55		0.041		F	18.00	18.01	0.132	0.543
	9.65		0.032		I	18.24	18.25	0.349	0.741
	9.98		0.021		K	18.55	18.56	0.299	1.040
	10.22		0.035		L	18.77		0.030	
R	10.43	10.40	0.096	0.395		18.86		0.032	
Y	11.15	11.15	0.162	0.521		19.06		0.024	
	11.34		0.037			19.29		0.032	
	11.57		0.026			19.46		0.030	
	11.71		0.043			19.57		0.038	
	11.84		0.026			19.71		0.057	

Saturday, March 31, 2012 11:25:07

120330_2A_bLG - Page 19 of 19



10.4 Edman Sequencing Data for phosphinothricin acetyltransferase (PAT) protein from Event SYHT0H2

N-terminal sequencing data sheet

Client / Job number: Syngenta / 23099
Sample number: 99740 + 99741
Performed by/date: CB 02Apr2012
Reviewed by/date: _____

Residue Number	Sequence		Comments
	PTH-AA	pmoles *	
1	S	19.7	No T1et observed in this cycle .
2	P	13.6	
3	E	15.3	
4	R	12.2	
5	R	18.3	
6	P	10.1	
7	V	10.5	
8	E	10.5	
9	I	2.6 9.0	CB 02Apr2012
10	R	8.4	
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			

* Calculation based on 10pmole containing standard.

Page 1 of 1

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SequencePro™

EPB 02Apr2012

SGS M-Scan Ltd
SGS M-Scan job: 23099
SGS M-Scan sample: 99740-41
Operator: EPS

SAMPLE INFORMATION

Sample Name:	SYHT0H2 purified PAT	Std Amount:	10.000 pmols
ID Code:	99740-41	Sample Amount:	0.000 pmols
		Detector Scale:	0.005 AUFS
Comments:			

SEQUENCER INFORMATION

Name:	PROCISE	Model Number:	492
Method:	Pulsed liquid PVDF	Cartridge:	A
Operator:	EPS		

CHEMICAL INFORMATION

R1	1111174	22 March, 2012	X3	0	01 September, 2001
R2	1105087	06 March, 2012	PTH Column	B120808034	28 March, 2012
R3	1012198	23 March, 2012	Solvent A	1112630	25 March, 2012
R4	1111134	25 March, 2012	Solvent B	1107354	23 March, 2012
R5	1011047	30 March, 2012	Premix	1104103	02 March, 2012
S1	0	01 September, 2001	Guard Column	0	01 January, 2002
S2	1109711	26 March, 2012	Cartridge Seals	0	01 January, 2002
S3	1112502	26 March, 2012	Glass Fiber Filter	0	01 January, 2002
S4	1201170	28 March, 2012	PTH standards	1007026	01 January, 2002
X1	0	01 September, 2001		12345678	01 January, 2002
X2	0	01 September, 2001	Total Cycles Count		01 January, 2002

Monday, April 02, 2012 09:38:00

120331_1A_99740_41 - 02Apr2012 08:54:52 - Page 1 of 14

SequencePro™

EPS 02Apr2012

SGS M-Scan Ltd
 SGS M-Scan job: 23099
 SGS M-Scan sample: 99740-41
 Operator: EPS

ORIGINAL METHOD TEMPLATE: C:\Program Files\AppliedBiosystems\Precise\SequencePro\Methods\Precise.met

CALIBRATION TABLE

COMPONENT	RTIME	RESPONSE	REFERENCE	INTERNAL STD	ABS WND	REL WND
Aspartic Acid	4.37	1.000	---	---	0.20	0.00
Asparagine	4.90	1.000	---	---	0.20	0.00
Serine	5.61	1.000	---	---	0.20	0.00
Glutamine	5.88	1.000	---	---	0.20	0.00
Threonine	6.18	1.000	---	---	0.20	0.00
Glycine	6.42	1.000	---	---	0.20	0.00
Glutamic Acid	6.88	1.000	---	---	0.20	0.00
Histidine	8.10	1.000	---	---	0.20	0.00
Alanine	8.84	1.000	---	---	0.20	0.00
Arginine	10.40	1.000	---	---	0.20	0.00
Tyrosine	11.15	1.000	---	---	0.20	0.00
Proline	13.52	1.000	---	---	0.20	0.00
Methionine	14.23	1.000	---	---	0.20	0.00
Valine	14.56	1.000	---	---	0.20	0.00
dptu	15.77	1.000	---	---	0.20	0.00
Tryptophan	16.65	1.000	---	---	0.20	0.00
Phenylalanine	17.52	1.000	---	---	0.20	0.00
Isoleucine	18.01	1.000	---	---	0.20	0.00
Lysine	18.25	1.000	---	---	0.20	0.00
Leucine	18.56	1.000	---	---	0.20	0.00

GLOBAL INTEGRATION EVENTS

EVENT	TIME	VALUE	EVENT	TIME	VALUE
Peak Detect Off	0.00	---	Valley to Valley Off	5.30	---
Valley to Valley On	3.90	---	Peak Detect Off	20.00	---
Peak Detect On	3.90	---			

INTEGRATION PARAMETERS

PEAK DETECTION PARAMETERS			
Bunching Factor	4	Noise Threshold:	0.954 μ Volts
Max Peaks	128	Area Threshold:	41.000 μ Volts
PEAK SEPARATION CRITERIA		EXPONENTIAL SKIM CRITERIA	
Width Ratio:	0.20	Peak Height Ratio:	5.00
Valley to Peak Ratio:	0.01	Adjusted Height Ratio:	4.00
Tangent Width:	1000.00	Valley Height Ratio:	3.00

SEQUENCE CALLING PARAMETERS

Use Pmol Heights, Allow Negative Background Off, Refine Data On			
SEQUENCE SCORING VALUES			
Raw Slope 1:	2.00	Raw Yield	1.00
Raw Slope 2:	1.00	Bkgd Yield	1.00
Bkgd Slope 1:	2.00	Lag Yield	1.00
Bkgd Slope 2:	1.00	Rep Yield	1.00
Max Slope:	1.50	Low Yield	1.00
Rule Book:	0.60	Bkgd Sensitivity	1.00
Dev Mult:	3.00		

Monday, April 02, 2012 09:38:00

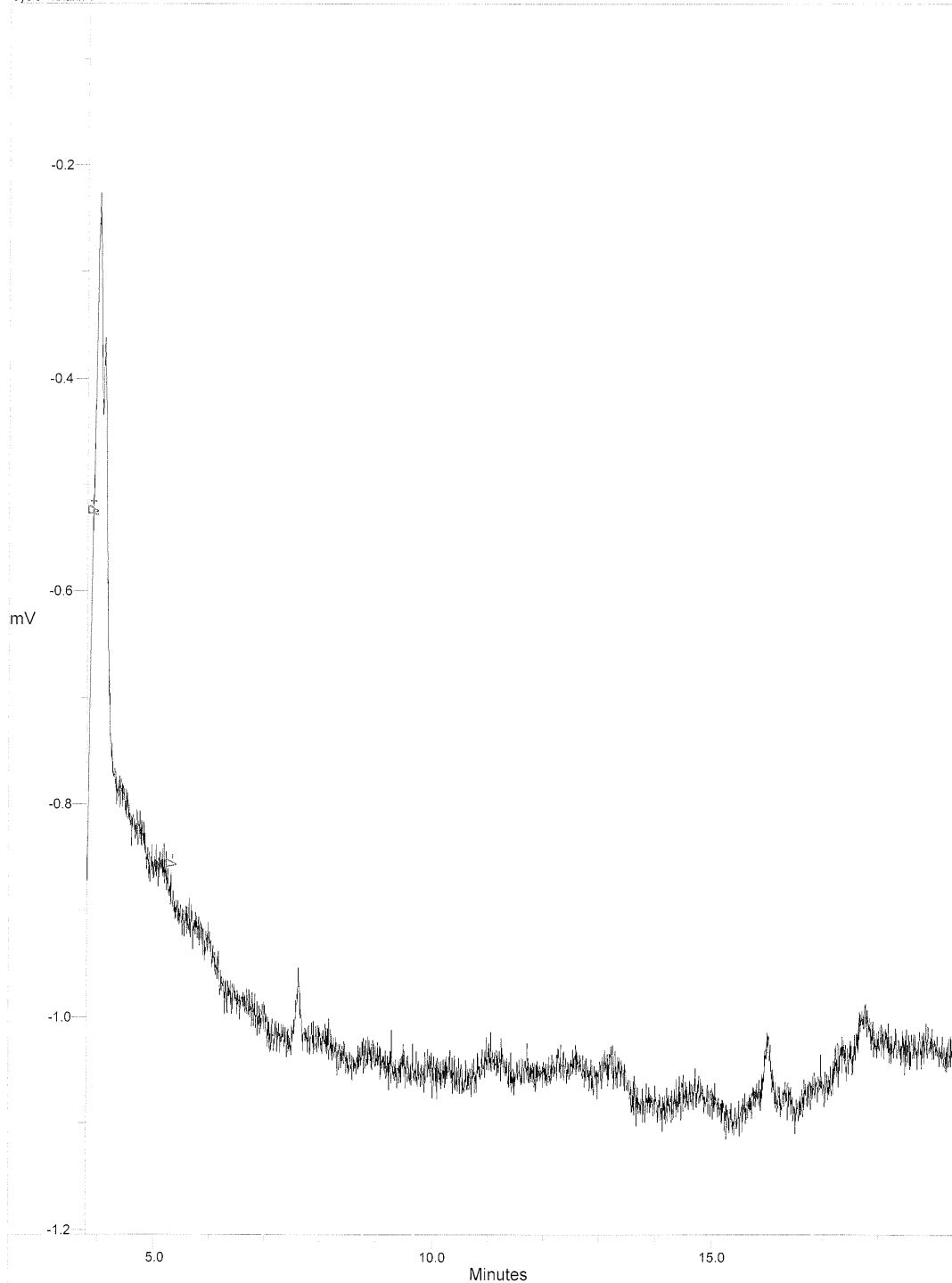
120331_1A_99740_41 - 02Apr2012 08:54:52 - Page 2 of 14

SequencePro™

EPS 02Apr2012

SGS M-Scan Ltd
SGS M-Scan job: 23099
SGS M-Scan sample: 99740-41
Operator: EPS

Cycle 1: Blank 1



Monday, April 02, 2012 09:38:00

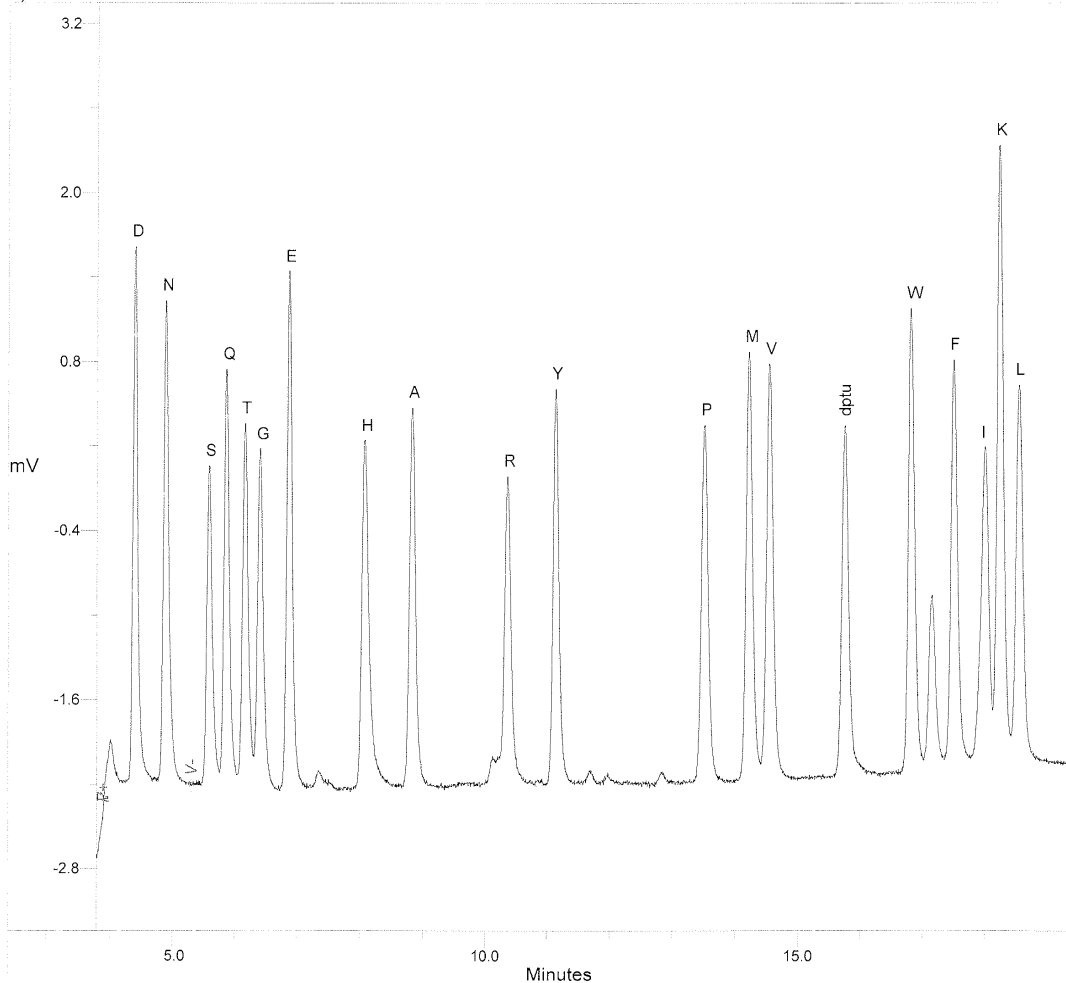
120331_1A_99740_41 - 02Apr2012 08:54:52 - Page 3 of 14

SequencePro™

02Apr2012

SGS M-Scan Ltd
SGS M-Scan job: 23099
SGS M-Scan sample: 99740-41
Operator: EPS

Cycle 2: Standard 1



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.02		0.513			11.98		0.076	
	4.39	4.39	3.806	10.000		12.12		0.029	
	4.70		0.034			12.29		0.036	
N	4.88	4.88	3.411	10.000		12.85		0.096	
	5.33		0.029			13.11		0.031	
S	5.58	5.58	2.256	10.000		13.19		0.032	
Q	5.86	5.86	2.905	10.000		13.29		0.031	
T	6.16	6.16	2.521	10.000	P	13.53	13.53	2.536	10.000
G	6.40	6.40	2.376	10.000	M	14.23	14.23	3.052	10.000
E	6.87	6.87	3.672	10.000	V	14.56	14.56	2.955	10.000
	7.16		0.040			15.20		0.034	
	7.34		0.121			15.50		0.021	
	7.82		0.038		dptu	15.77	15.77	2.497	10.000
H	8.07	8.07	2.473	10.000		16.48		0.034	
A	8.83	8.83	2.698	10.000		16.60		0.032	
	9.30		0.040		W	16.83	16.83	3.279	10.000
	9.35		0.025			17.17		1.200	
	9.50		0.026		F	17.52	17.52	2.841	10.000
	9.59		0.034		I	18.02	18.02	2.213	10.000
	9.72		0.028		K	18.25	18.25	4.364	10.000
	10.13		0.212		L	18.55	18.55	2.663	10.000
R	10.37	10.37	2.187	10.000		18.99		0.036	
	10.86		0.046			19.06		0.023	
	10.93		0.049			19.16		0.031	
Y	11.15	11.15	2.813	10.000		19.38		0.037	
	11.69		0.100			19.75		0.029	

Monday, April 02, 2012 09:38:00

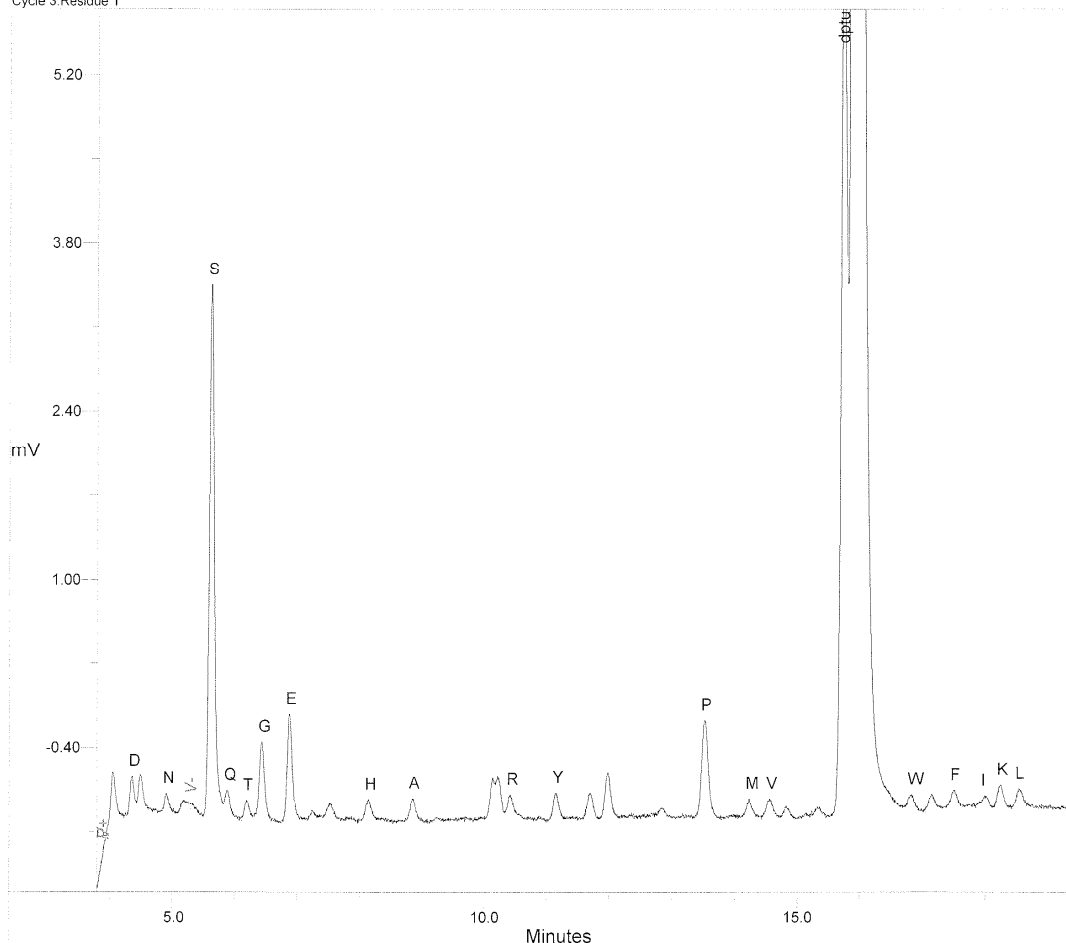
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SequencePro™

EP 02Apr12

SGS M-Scan Ltd
SGS M-Scan job: 23099
SGS M-Scan sample: 99740-41
Operator: EPS

Cycle 3:Residue 1



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.05		0.624			12.36		0.051	
	4.36	4.39	0.279	0.734		12.56		0.035	
	4.49		0.261			12.62		0.029	
N	4.91	4.88	0.175	0.514		12.84		0.084	
	5.19		0.082			13.17		0.044	
S	5.63	5.58	4.434	19.655	P	13.52	13.53	0.820	3.233
Q	5.89	5.86	0.221	0.762		13.88		0.022	
T	6.20	6.16	0.175	0.693		13.96		0.022	
G	6.44	6.40	0.663	2.791	M	14.23	14.23	0.175	0.575
E	6.88	6.87	0.899	2.448	V	14.57	14.56	0.178	0.601
	7.23		0.089			14.82		0.111	
	7.53		0.149			15.15		0.050	
	7.78		0.031			15.36		0.094	
	7.98		0.039		dplu	15.76	15.77	7.323	29.322
H	8.13	8.07	0.191	0.772		15.98		80.133	
	8.62		0.029		W	16.83	16.83	0.133	0.406
A	8.85	8.83	0.201	0.746		17.00		0.027	
	9.22		0.036			17.15		0.147	
	9.34		0.026		F	17.51	17.52	0.153	0.538
	9.96		0.034			17.75		0.028	
	10.14		0.349			17.82		0.040	
	10.22		0.356		I	18.00	18.02	0.109	0.495
R	10.42	10.37	0.186	0.849	K	18.25	18.25	0.198	0.455
	10.88		0.051		L	18.54	18.55	0.159	0.596
Y	11.16	11.15	0.239	0.850		18.75		0.029	
	11.33		0.025			19.25		0.033	
	11.70		0.233			19.50		0.084	
	11.99		0.394			19.58		0.115	
	12.20		0.038			19.73		0.226	

Monday, April 02, 2012 09:38:00

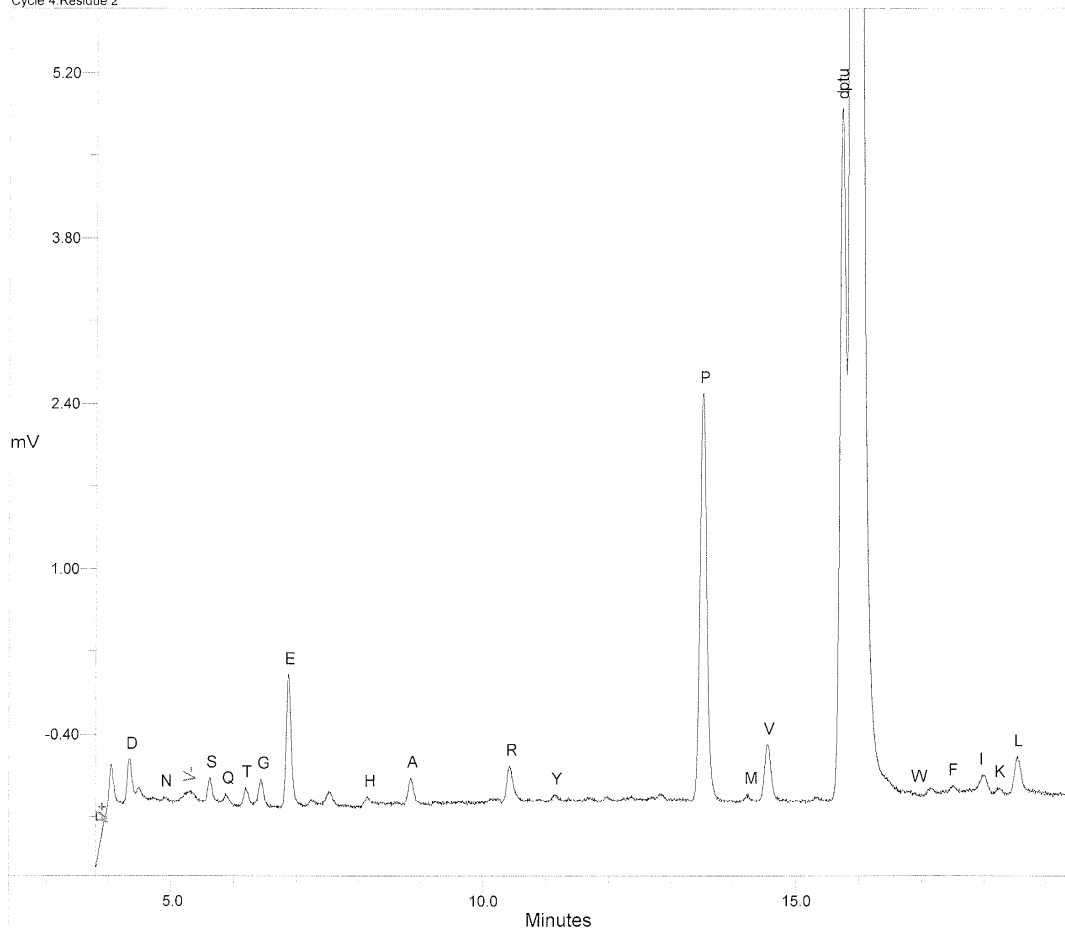
120331_1A_99740_41 - 02Apr2012 08:54:52 - Page 5 of 14

SequencePro™

EPS 02Apr2012

SGS M-Scan Ltd
SGS M-Scan job: 23099
SGS M-Scan sample: 99740-41
Operator: EPS

Cycle 4: Residue 2



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.05		0.533			11.97		0.051	
	4.34		0.344			12.15		0.023	
	4.49	4.39	0.074	0.904		12.24		0.036	
	4.71		0.040			12.37		0.047	
N	4.89	4.88	0.058	0.169		12.71		0.046	
	5.31		0.096			12.83		0.073	
S	5.62	5.58	0.226	1.001		13.00		0.028	
Q	5.87	5.86	0.109	0.375		13.13		0.035	
T	6.18	6.16	0.179	0.709	P	13.52	13.53	3.455	13.623
G	6.42	6.40	0.255	1.072		13.86		0.032	
E	6.87	6.87	1.136	3.094	M	14.24	14.23	0.074	0.242
	7.24		0.079		V	14.55	14.56	0.494	1.671
	7.52		0.147			14.92		0.027	
	8.00		0.024			15.10		0.041	
H	8.14	8.07	0.096	0.387		15.30		0.051	
	8.51		0.039			15.49		0.025	
	8.62		0.040		dptu	15.76	15.77	5.817	23.291
A	8.84	8.83	0.241	0.893		15.97		69.139	
	9.68		0.045		W	16.90	16.83	0.037	0.113
	9.90		0.033			17.14		0.067	
	10.14		0.059		F	17.49	17.52	0.067	0.236
	10.17		0.060		I	17.98	18.02	0.159	0.720
R	10.42	10.37	0.324	1.484	K	18.23	18.25	0.068	0.155
	10.74		0.042		L	18.54	18.55	0.319	1.198
	10.85		0.031			18.89		0.040	
	11.04		0.024			19.12		0.034	
Y	11.15	11.15	0.074	0.263		19.23		0.048	
	11.36		0.050			19.39		0.029	
	11.54		0.029			19.45		0.024	
	11.61		0.035			19.72		0.158	

Monday, April 02, 2012 09:38:00

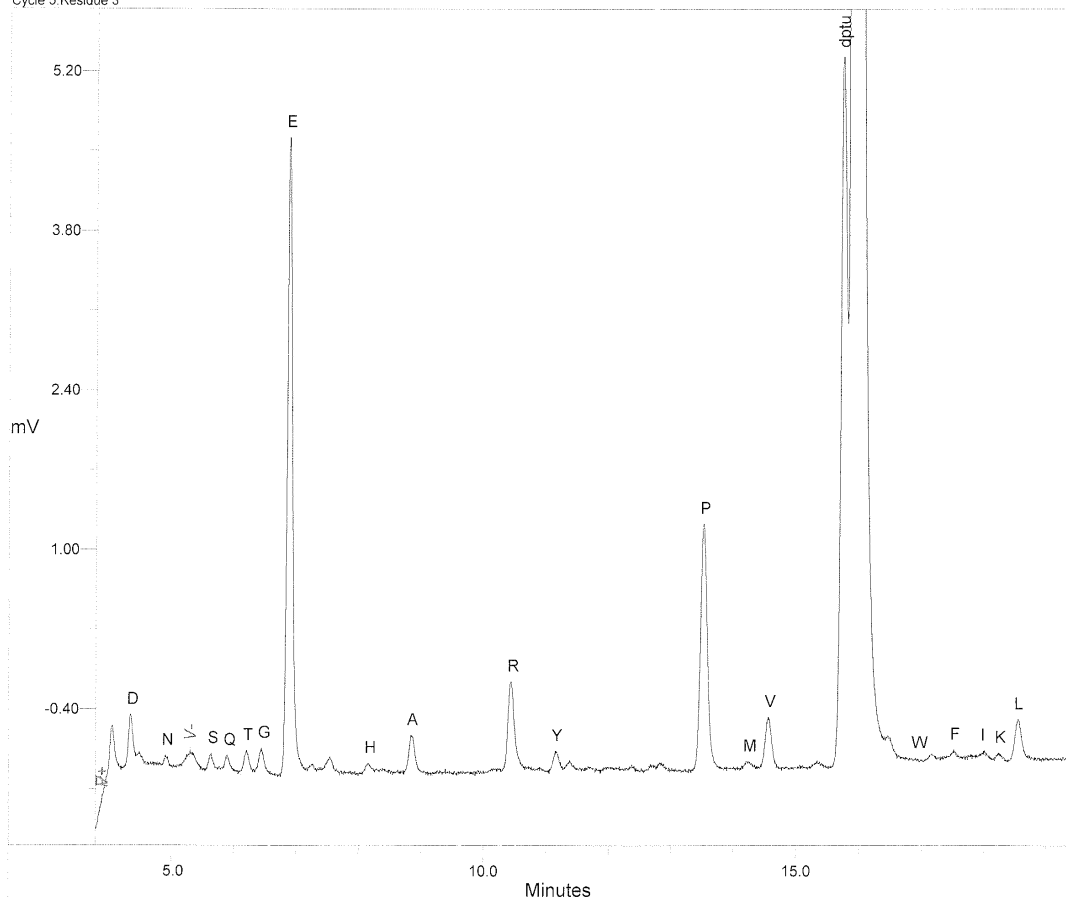
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ES 02 Apr 2012

SGS M-Scan Ltd
SGS M-Scan job: 23099
SGS M-Scan sample: 99740-41
Operator: EPS

Cycle 5: Residue 3



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.06		0.577			11.99		0.036	
	4.35	4.39	0.413	1.085		12.30		0.028	
N	4.49		0.085			12.38		0.058	
	4.90	4.88	0.107	0.315		12.68		0.073	
S	5.30		0.170			12.82		0.065	
Q	5.64	5.58	0.166	0.737		13.02		0.034	
T	5.89	5.86	0.160	0.550		13.29		0.038	
G	6.20	6.16	0.218	0.863	P	13.53	13.53	2.169	8.550
E	6.44	6.40	0.246	1.036		13.89		0.035	
	6.88	6.87	5.604	15.258		14.01		0.034	
	7.26		0.084		M	14.23	14.23	0.081	0.267
	7.54		0.136		V	14.57	14.56	0.465	1.573
H	7.72		0.043			14.77		0.020	
	8.15	8.07	0.103	0.415		15.11		0.032	
	8.29		0.040			15.35		0.075	
	8.38		0.039		dptu	15.78	15.77	6.238	24.979
A	8.63		0.041			15.98		73.882	
	8.84	8.83	0.340	1.261		16.46		0.195	
	9.19		0.030		W	16.90	16.83	0.033	0.102
	9.26		0.032			16.98		0.024	
	9.39		0.047			17.16		0.066	
	9.54		0.033		F	17.52	17.52	0.075	0.263
	9.63		0.036			17.79		0.050	
	9.68		0.038			17.88		0.050	
	9.85		0.040		I	18.02	18.02	0.093	0.420
	9.91		0.045		K	18.24	18.25	0.079	0.182
R	10.23		0.042		L	18.55	18.55	0.367	1.380
	10.44	10.37	0.791	3.615		18.90		0.033	
	10.90		0.032			19.00		0.035	
Y	11.15	11.15	0.199	0.708		19.08		0.023	
	11.38		0.111			19.51		0.032	
	11.68		0.051			19.75		0.158	

Monday, April 02, 2012 09:38:00

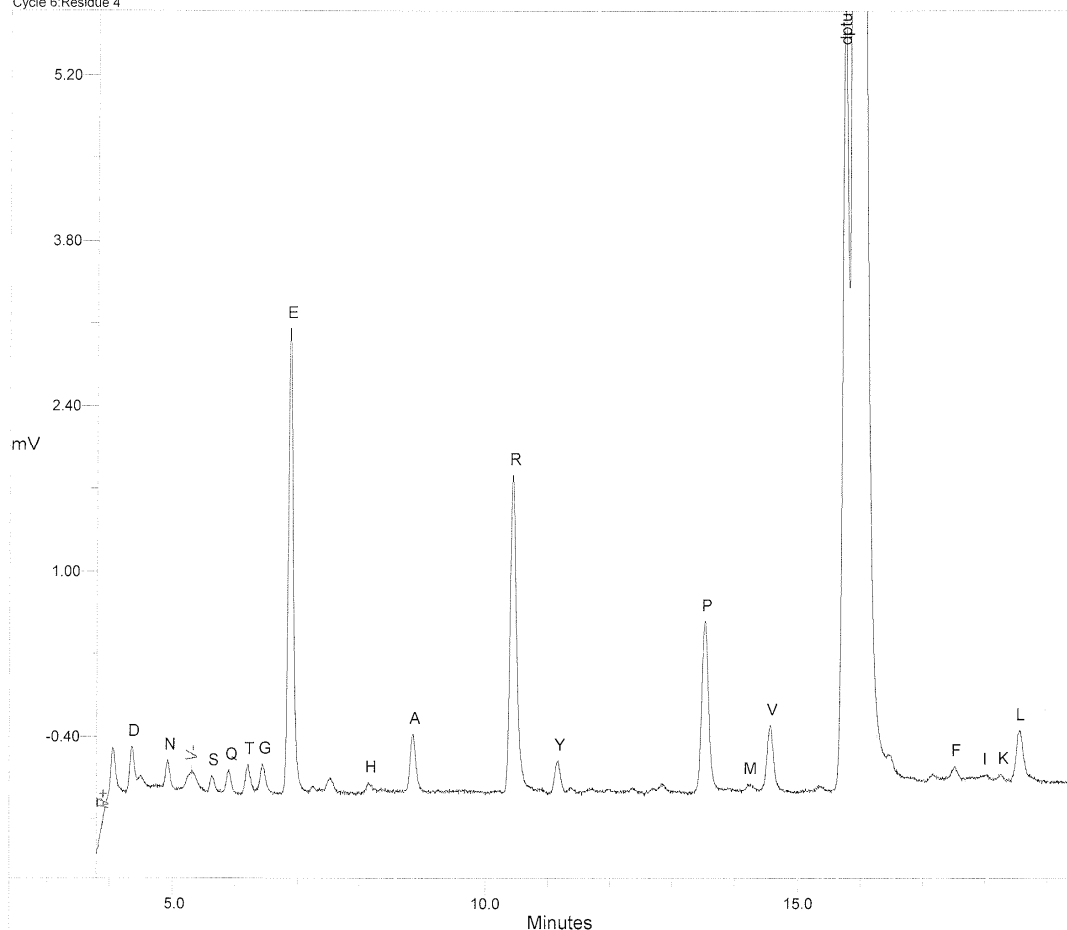
120331_1A_99740_41 - 02Apr2012 08:54:52 - Page 7 of 14

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EPS 02Apr2012

SGS M-Scan Ltd
SGS M-Scan job: 23099
SGS M-Scan sample: 99740-41
Operator: EPS

Cycle 6:Residue 4



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.05		0.575			12.37		0.056	
	4.35	4.39	0.345	0.908		12.60		0.051	
	4.49		0.082			12.69		0.065	
N	4.92	4.88	0.253	0.743		12.83		0.096	
	5.09		0.032			13.01		0.035	
	5.31		0.171		P	13.53	13.53	1.455	5.738
S	5.62	5.58	0.152	0.672		14.09		0.036	
Q	5.90	5.86	0.214	0.737	M	14.20	14.23	0.045	0.147
T	6.20	6.16	0.265	1.053	V	14.57	14.56	0.559	1.891
G	6.43	6.40	0.268	1.129		14.82		0.021	
E	6.88	6.87	3.947	10.747		14.95		0.025	
	7.26		0.070			15.08		0.031	
	7.35		0.049			15.34		0.067	
	7.53		0.136		dptu	15.77	15.77	6.616	26.492
	7.99		0.035			15.98		84.445	
H	8.15	8.07	0.087	0.353		16.44		0.201	
	8.35		0.036			17.16		0.059	
	8.61		0.034		F	17.52	17.52	0.128	0.451
A	8.85	8.83	0.501	1.856		17.66		0.033	
	9.11		0.029			17.75		0.045	
	9.25		0.034		I	18.02	18.02	0.063	0.285
	9.64		0.040		K	18.26	18.25	0.071	0.164
	9.82		0.029		L	18.55	18.55	0.427	1.602
	10.05		0.028			18.93		0.037	
R	10.44	10.37	2.674	12.224		18.99		0.030	
Y	11.17	11.15	0.291	1.036		19.14		0.028	
	11.37		0.062			19.35		0.027	
	11.68		0.027			19.40		0.034	
	11.90		0.023			19.63		0.057	
	11.98		0.029			19.74		0.156	

Monday, April 02, 2012 09:38:00

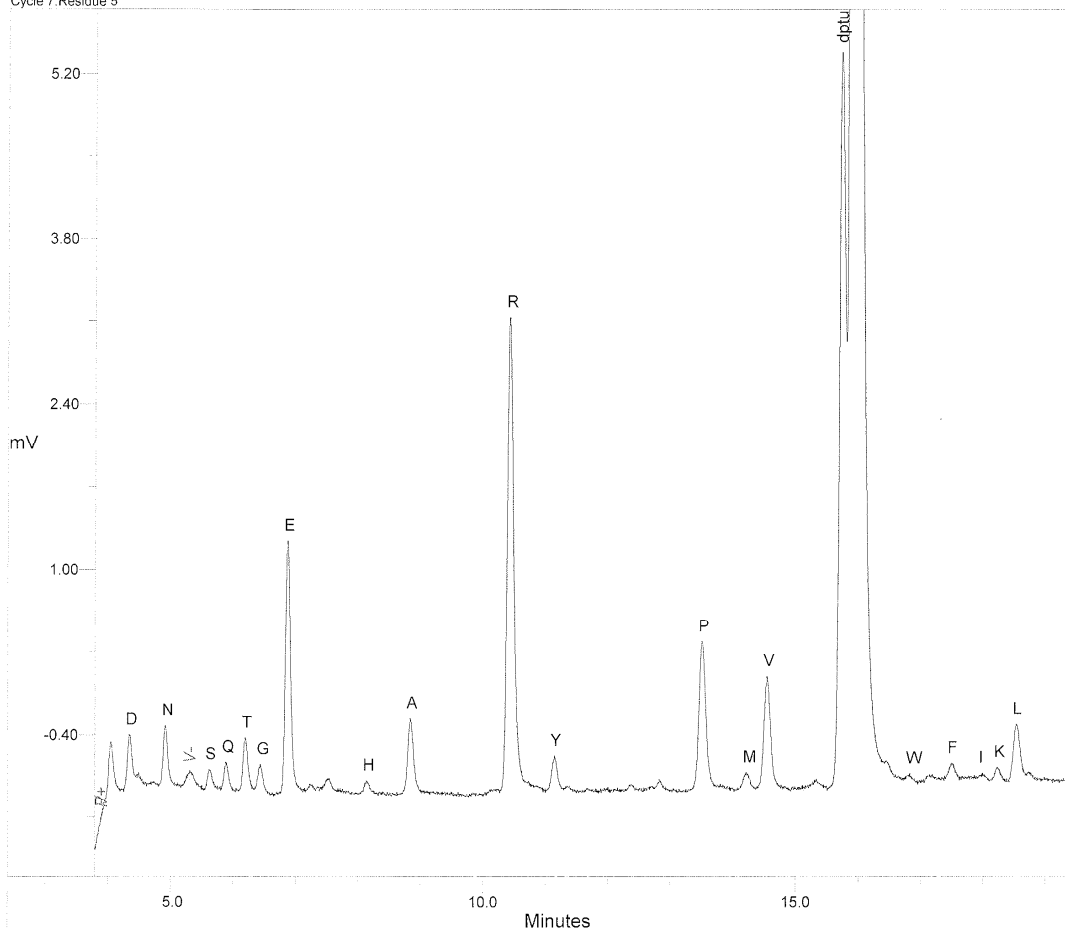
120331_1A_99740_41 - 02Apr2012 08:54:52 - Page 8 of 14

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EPS 02Apr2012

SGS M-Scan Ltd
SGS M-Scan job: 23099
SGS M-Scan sample: 99740-41
Operator: EPS

Cycle 7: Residue 5



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.05		0.598			11.51		0.028	
	4.34	4.39	0.429	1.127		11.67		0.036	
	4.73		0.043			12.00		0.049	
N	4.91	4.86	0.512	1.500		12.16		0.036	
	5.31		0.152			12.25		0.023	
S	5.61	5.58	0.181	0.804		12.38		0.054	
Q	5.88	5.86	0.254	0.876		12.70		0.050	
	6.05		0.037			12.83		0.111	
T	6.19	6.16	0.473	1.875		13.05		0.038	
G	6.43	6.40	0.259	1.092		13.25	13.53	1.249	4.923
E	6.87	6.87	2.146	5.844	P	13.52		0.031	
	7.22		0.076			13.80		0.030	
	7.36		0.050			13.92		0.159	0.522
	7.54		0.130		M	14.23	14.23	0.962	3.256
	7.72		0.041		V	14.55	14.56	0.038	
H	8.14	8.07	0.127	0.514		15.00		0.099	
	8.40		0.038			15.34		6.194	24.801
	8.63		0.030		dptu	15.76	15.77	63.258	
A	8.84	8.83	0.645	2.390		15.97		0.075	0.229
	9.03		0.029		W	16.82	16.83	0.044	
	9.21		0.031			17.18		0.150	0.528
	9.27		0.027		F	17.49	17.52	0.029	
	9.39		0.028			17.77		0.040	
	9.60		0.035			17.87		0.068	0.306
	9.84		0.038		I	17.99	18.02	0.122	0.279
	9.95		0.026		K	18.23	18.25	0.465	1.746
	10.21		0.044		L	18.53	18.55	0.037	
R	10.44	10.37	4.000	18.288		19.13		0.032	
Y	11.14	11.15	0.312	1.108		19.30		0.133	
	11.39		0.054			19.72			

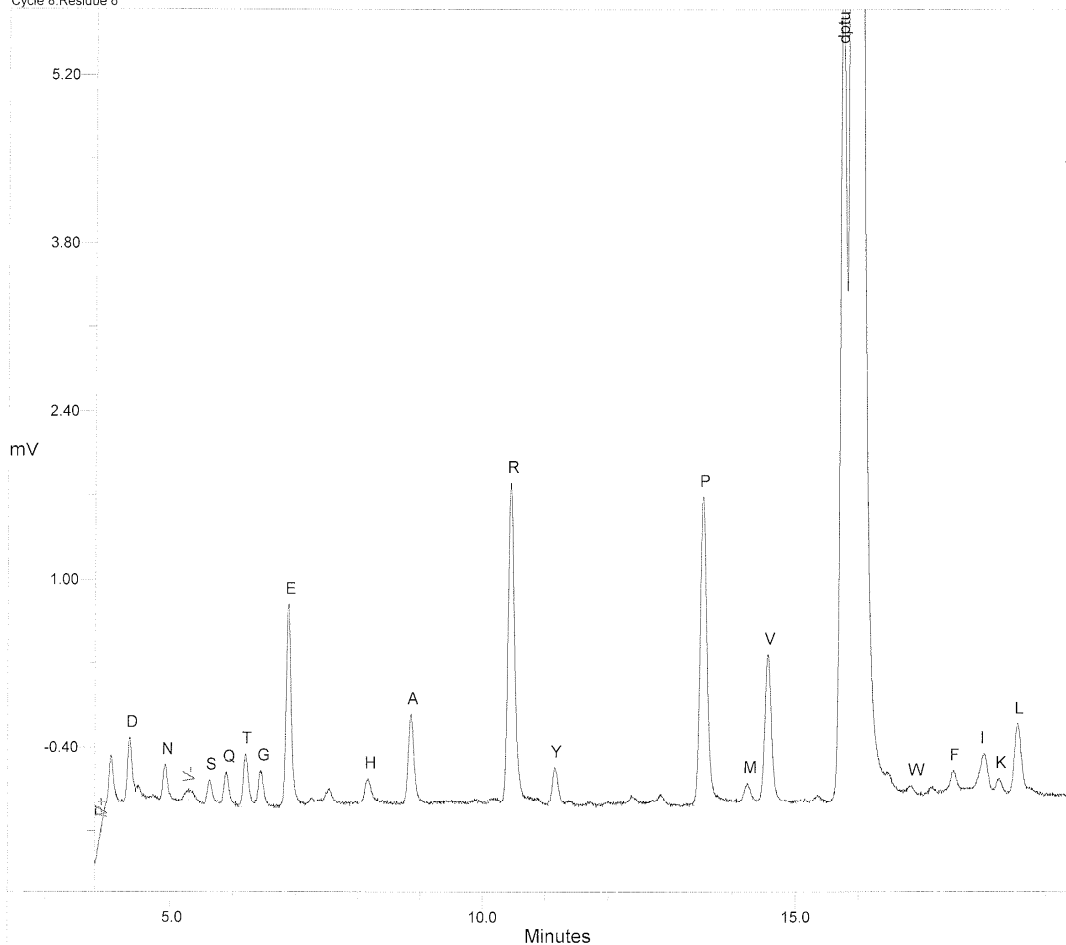
Monday, April 02, 2012 09:38:00

120331_1A_99740_41 - 02Apr2012 08:54:52 - Page 9 of 14

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SGS M-Scan Ltd
SGS M-Scan job: 230999
SGS M-Scan sample: 99740-41
Operator: EPS

Cycle 8: Residue 6



PEAK ID	R. TIME (mins)	C. TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R. TIME (mins)	C. TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.05		0.558		Y	11.15	11.15	0.328	1.167
	4.35	4.39	0.500	1.313		11.38		0.057	
	4.49		0.075			11.49		0.033	
N	4.92	4.88	0.302	0.885		12.01		0.047	
	5.30		0.040			12.20		0.043	
S	5.63	5.58	0.214	0.947		12.37		0.090	
Q	5.90	5.86	0.292	1.005		12.71		0.045	
T	6.20	6.16	0.453	1.796		12.85		0.094	
G	6.44	6.40	0.316	1.329	P	13.53	13.53	2.562	10.102
E	6.88	6.87	1.704	4.640	M	14.24	14.23	0.174	0.570
	7.26		0.071		V	14.57	14.56	1.246	4.217
	7.42		0.059			14.84		0.034	
	7.55		0.138			15.00		0.034	
	7.75		0.027			15.15		0.048	
	7.85		0.041			15.36		0.074	
	7.89		0.040		dptu	15.78	15.77	6.925	27.731
H	8.16	8.07	0.209	0.844		15.98		70.699	
	8.63		0.036			16.45		0.213	
A	8.85	8.83	0.762	2.825	W	16.85	16.83	0.115	0.352
	9.22		0.030			17.18		0.099	
	9.37		0.036		F	17.52	17.52	0.221	0.779
	9.51		0.031			17.68		0.077	
	9.66		0.028		I	18.02	18.02	0.349	1.576
	9.88		0.049		K	18.25	18.25	0.136	0.313
	10.05		0.031		L	18.56	18.55	0.576	2.163
	10.17		0.034			18.91		0.034	
R	10.45	10.37	2.653	12.127		19.02		0.030	
	10.77		0.031			19.30		0.026	
	10.86		0.044			19.71		0.036	

Monday, April 02, 2012 09:38:00

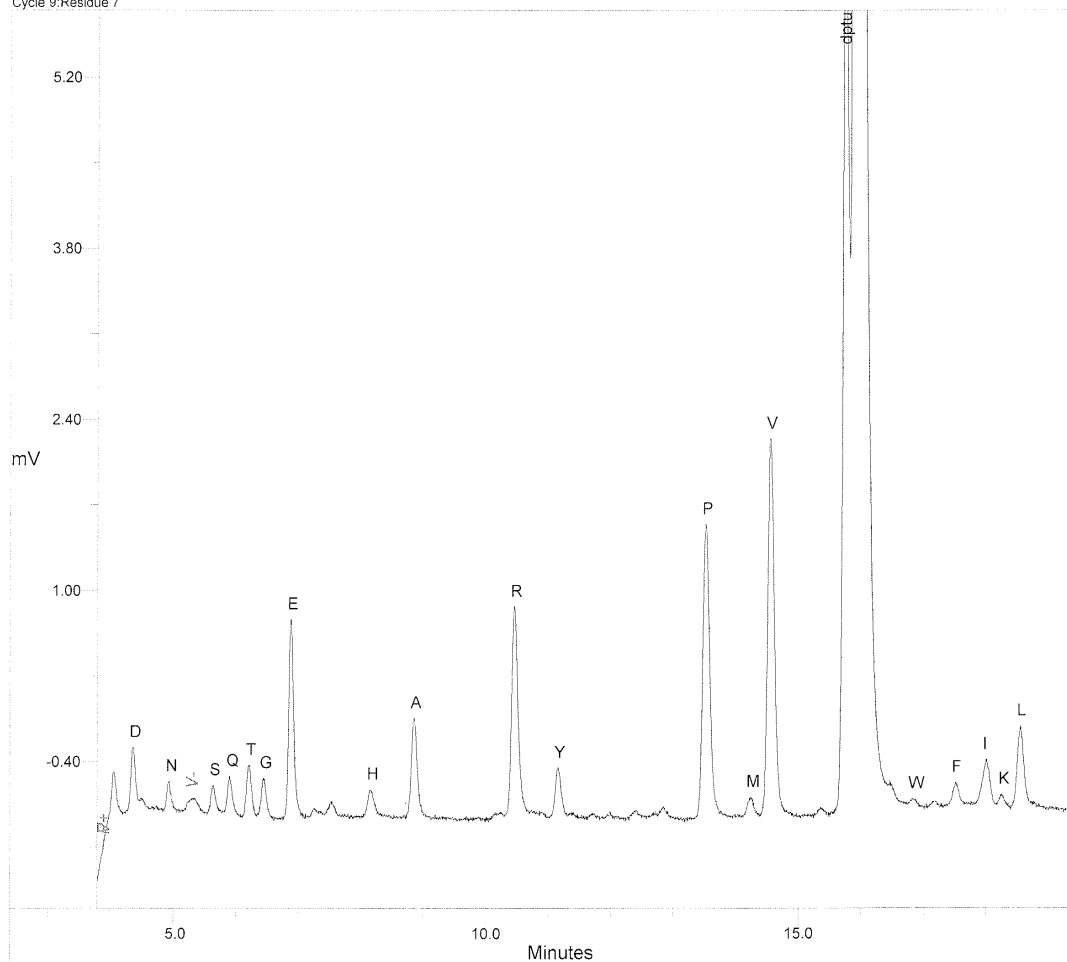
120331_1A_99740_41 - 02Apr2012 08:54:52 - Page 10 of 14

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SGS M-Scan Ltd
SGS M-Scan job: 23099
SGS M-Scan sample: 99740-41
Operator: EPS

Cycle 9: Residue 7



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.05		0.544			12.00		0.061	
	4.36	4.39	0.491	1.291		12.23		0.032	
	4.48		0.047			12.42		0.073	
N	4.93	4.88	0.264	0.775		12.72		0.063	
	5.35		0.149			12.86		0.101	
S	5.64	5.58	0.280	1.151	P	13.54	13.53	2.402	9.472
Q	5.90	5.86	0.345	1.189		13.98		0.028	
T	6.22	6.16	0.448	1.777	M	14.24	14.23	0.174	0.569
G	6.44	6.40	0.352	1.484	V	14.57	14.56	3.096	10.477
E	6.89	6.87	1.647	4.486		14.98		0.039	
	7.27		0.083			15.10		0.031	
	7.33		0.057			15.36		0.086	
	7.54		0.126		dptu	15.78	15.77	7.373	29.524
	7.85		0.033			15.99		74.526	
H	8.16	8.07	0.236	0.953		16.46		0.176	
	8.70		0.033		W	16.82	16.83	0.068	0.206
A	8.86	8.83	0.825	3.059		17.18		0.067	
	9.47		0.031		F	17.52	17.52	0.205	0.722
	9.70		0.043		I	18.02	18.02	0.397	1.792
	9.89		0.038		K	18.27	18.25	0.125	0.287
	9.95		0.036		L	18.56	18.55	0.673	2.527
	10.15		0.037			18.85		0.037	
R	10.46	10.37	1.726	7.892		19.22		0.028	
	10.74		0.037			19.38		0.067	
	10.86		0.038			19.46		0.093	
Y	11.16	11.15	0.420	1.493		19.57		0.143	
	11.39		0.059			19.76		0.251	
	11.71		0.060						

Monday, April 02, 2012 09:38:00

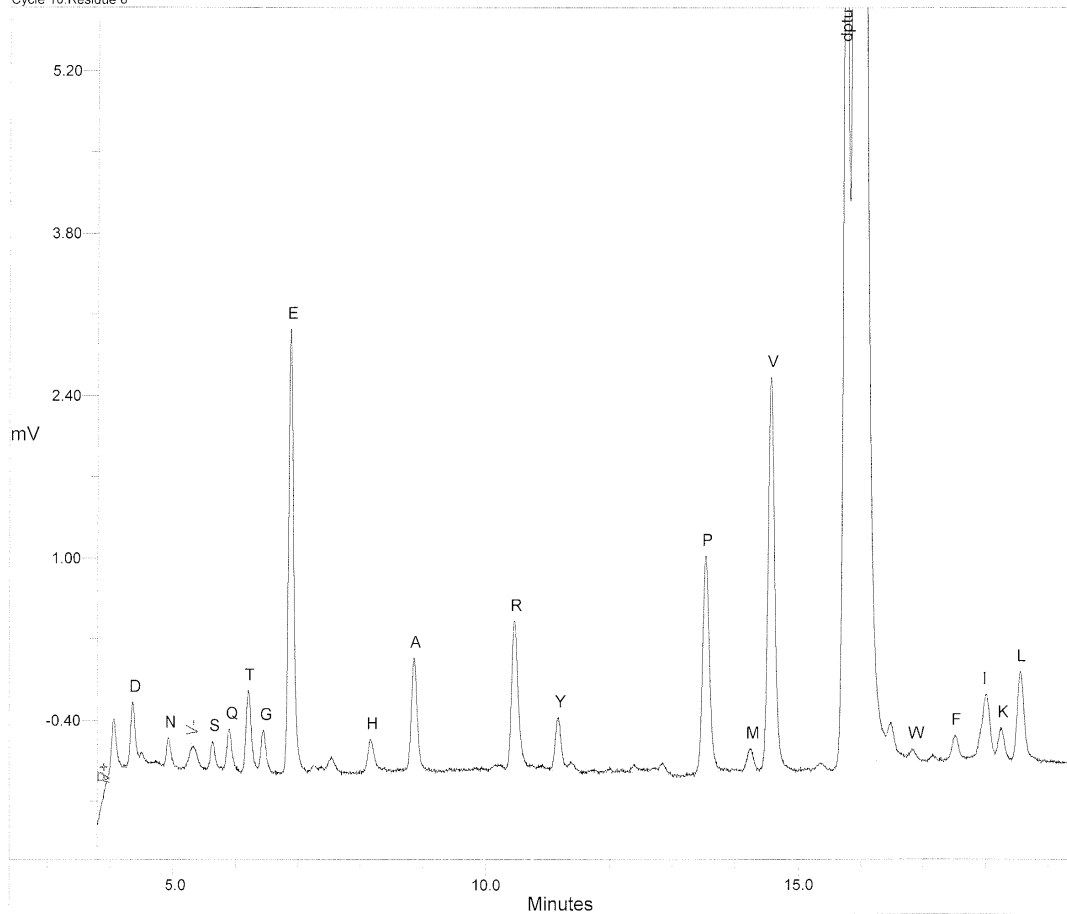
120331_1A_99740_41 - 02Apr2012 08:54:52 - Page 11 of 14

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EP 02Apr2012

SGS M-Scan Ltd
SGS M-Scan job: 230999
SGS M-Scan sample: 99740-41
Operator: EPS

Cycle 10: Residue 8



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.08		0.600			11.73		0.031	
	4.35	4.39	0.524	1.376		11.85		0.030	
	4.49		0.062			12.00		0.062	
	4.71		0.040			12.12		0.041	
N	4.92	4.88	0.255	0.747		12.29		0.022	
	5.32		0.217			12.38		0.067	
	5.63	5.58	0.268	1.190		12.73		0.057	
	5.90	5.86	0.380	1.307		12.84		0.111	
Q	6.20	6.16	0.716	2.839		13.08		0.030	
	6.45	6.40	0.376	1.583		13.23		0.041	
	6.88	6.87	3.841	10.459	P	13.53	13.53	1.886	7.436
	7.25		0.050			13.82		0.030	
T	7.38		0.053			13.95		0.029	
	7.53		0.149			14.23	14.23	0.205	0.671
G	7.70		0.041		M	14.57	14.56	3.398	11.498
	7.83		0.029			15.15		0.036	
	8.15	8.07	0.303	1.224		15.36		0.073	
	8.39		0.054			15.78	15.77	8.075	32.335
E	8.47		0.038			15.98		80.677	
	8.59		0.044		V	16.48		0.237	
	8.85	8.83	0.987	3.657		16.82	16.83	0.097	
	9.28		0.026			17.15		0.051	
H	9.43		0.039			17.52	17.52	0.235	0.827
	9.53		0.031		F	18.02	18.02	0.585	2.644
	9.85		0.036			18.25	18.25	0.299	0.686
	10.15		0.064			18.55	18.55	0.778	2.922
A	10.20		0.065			19.18		0.031	
	10.45	10.37	1.287	5.882		19.33		0.031	
	10.91		0.047			19.40		0.029	
	11.17	11.15	0.473	1.683		19.71		0.029	
R	11.37		0.102						
Y									

Monday, April 02, 2012 09:38:00

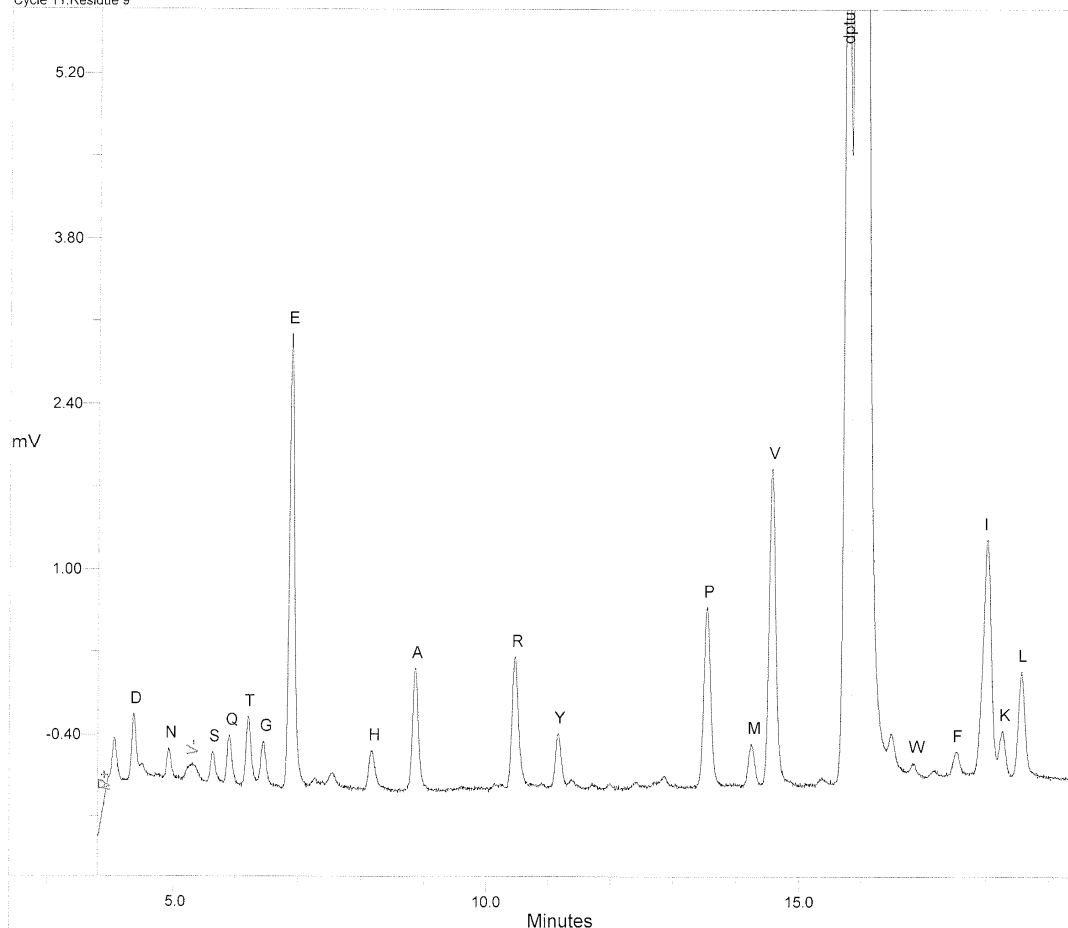
120331_1A_99740_41 - 02Apr2012 08:54:52 - Page 12 of 14

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CB 02Apr12

SGS M-Scan Ltd
SGS M-Scan job: 23099
SGS M-Scan sample: 99740-41
Operator: EPS

Cycle 11:Residue 9



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.06		0.565			11.71		0.065	
	4.36	4.39	0.511	1.342		12.00		0.062	
	4.49		0.050			12.14		0.047	
	4.70		0.041			12.43		0.057	
N	4.92	4.88	0.261	0.766		12.70		0.056	
	5.30		0.155			12.87		0.094	
S	5.63	5.58	0.270	1.199		13.03		0.036	
Q	5.90	5.86	0.427	1.469		13.28		0.030	
T	6.20	6.16	0.598	2.374		13.34		0.031	
G	6.45	6.40	0.396	1.666	P	13.54	13.53	1.530	6.033
E	6.89	6.87	3.846	10.471	M	14.24	14.23	0.368	1.205
	7.27		0.058		V	14.57	14.56	2.673	9.046
	7.39		0.039			15.03		0.032	
	7.53		0.119			15.11		0.032	
	7.96		0.035			15.18		0.028	
H	8.18	8.07	0.348	1.407		15.37		0.083	
	8.62		0.037		dptu	15.78	15.77	8.671	34.720
A	8.86	8.83	1.046	3.877		15.98		87.389	
	9.44		0.030			16.47		0.389	
	9.60		0.037		W	16.82	16.83	0.147	0.448
	9.74		0.028			17.19		0.042	
	9.81		0.044		F	17.52	17.52	0.207	0.729
	10.02		0.044			17.79		0.036	
	10.14		0.068		I	18.02	18.02	1.999	9.032
	10.23		0.051		K	18.26	18.25	0.369	0.845
R	10.47	10.37	1.094	5.000	L	18.56	18.55	0.894	3.357
	10.72		0.032			19.04		0.034	
	10.90		0.045			19.26		0.036	
Y	11.16	11.15	0.469	1.668		19.52		0.037	
	11.38		0.088						

Monday, April 02, 2012 09:38:00

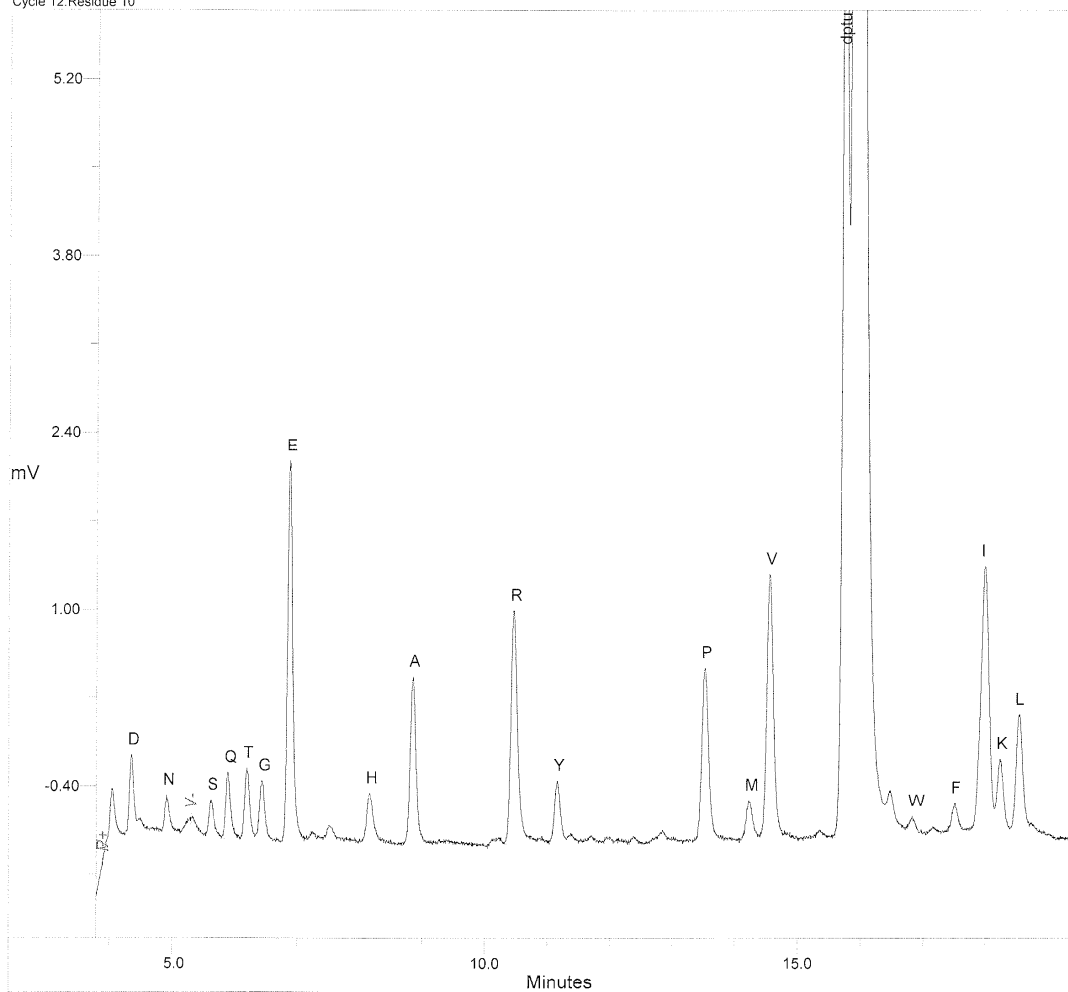
120331_1A_99740_41 - 02Apr2012 08:54:52 - Page 13 of 14

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EPS 02Apr2012

SGS M-Scan Ltd
SGS M-Scan job: 23099
SGS M-Scan sample: 99740-41
Operator: EPS

Cycle 12: Residue 10



PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT	PEAK ID	R.TIME (mins)	C.TIME (mins)	HEIGHT (mV)	PMOL HT
D	4.04		0.584			12.39		0.066	
	4.35	4.39	0.585	1.537		12.60		0.020	
	4.49		0.049			12.85		0.086	
N	4.91	4.88	0.304	0.890		13.15		0.030	
	5.33		0.152		P	13.53	13.53	1.369	5.397
	5.62	5.58	0.306	1.359	M	14.23	14.23	0.333	1.090
Q	5.89	5.86	0.535	1.840	V	14.57	14.56	2.106	7.126
T	6.20	6.16	0.575	2.281		14.80		0.052	
G	6.44	6.40	0.482	2.028		14.98		0.030	
E	6.88	6.87	3.022	8.229		15.07		0.028	
	7.23		0.075			15.20		0.041	
	7.33		0.039			15.36		0.069	
	7.52		0.115		dptu	15.77	15.77	8.239	32.991
H	8.16	8.07	0.393	1.590		15.98		72.842	
A	8.35	8.83	1.332	4.938		16.48		0.306	
	9.19		0.028		W	16.83	16.83	0.121	0.368
	9.81		0.028			17.03		0.025	
	10.19		0.031			17.16		0.084	
R	10.47	10.37	1.847	8.443	F	17.52	17.52	0.241	0.847
Y	11.16	11.15	0.504	1.791	I	18.02	18.02	2.102	9.497
	11.38		0.076		K	18.25	18.25	0.569	1.303
	11.54		0.031		L	18.55	18.55	0.902	3.385
	11.61		0.028			19.21		0.032	
	11.70		0.045			19.39		0.029	
	11.96		0.037			19.51		0.037	
	12.13		0.035			19.75		0.051	

Monday, April 02, 2012 09:38:00

120331_1A_99740_41 - 02Apr2012 08:54:52 - Page 14 of 14