

**CERTIFICATE OF ANALYSIS FOR THE TEST/REFERENCE/CONTROL SUBSTANCE:
ARYLOXYALKANOATE DIOXYGENASE (ABBREVIATION: AAD-12) – TSN030732
AMENDED REPORT**

TITLE OBJECTIVE

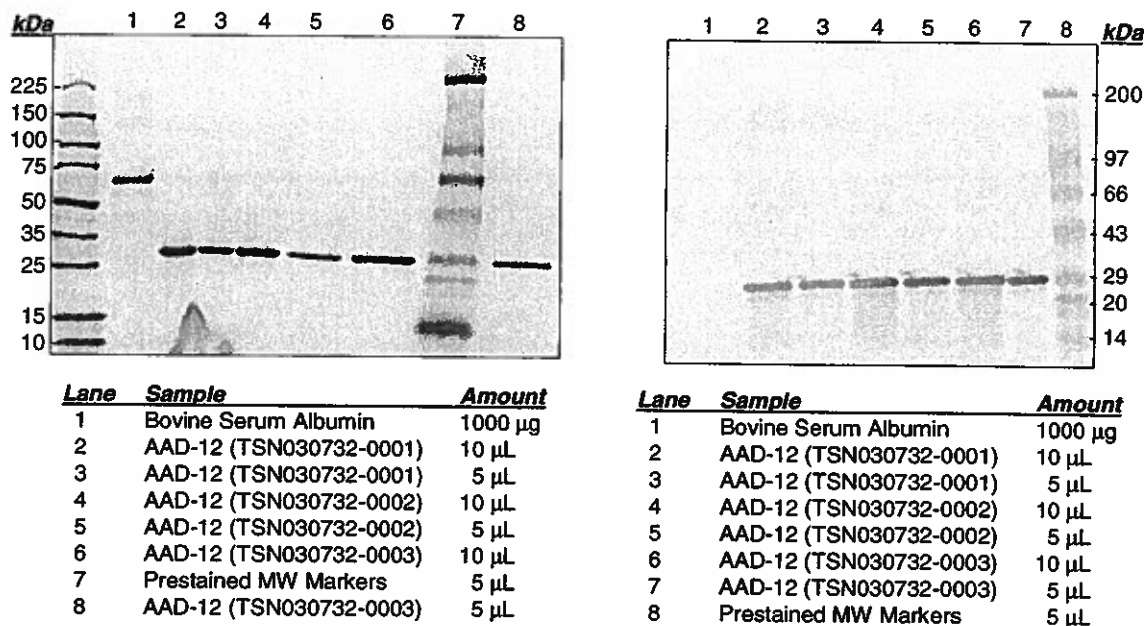
Determination of the purity and/or identity of the following test/reference/control substance for use in a study.

	TEST/REFERENCE/CONTROL SUBSTANCE
LOTS:	Lot 466-026: TSN030732-0001 Lot 466-028A: TSN030732-0002 Lot 466-028B: TSN030732-0003
DESCRIPTION	Aryloxyalkanoate dioxygenase (abbreviation: AAD-12) expressed in recombinant <i>Pseudomonas fluorescens</i> strain DC579 (derived from host MB324 strain by transformation with pMYC1803 expression vector).
MOLECULAR WEIGHT	Approximately 31.7 kDa
AMINO ACID SEQUENCE OF AAD-12	¹ MAQTTLQITPTGATLGATVTGVHLATLDDAGFAALHAAWLQHALLIFP GQHLSNDQQITFAKRFGAIERIGGGDIVAISNVKADGTVRQHSAPWD DMMKVIVGNMAWHADSTYMPVMAQGAVFSAEVVPAVGGRTCFADM RAAYDALDEATRALVHQRSARHSLVYSQSKLGHVQQAGSAYIGYGM TTATPLRPLVKVHPETGRPSLLIGRHAHAIPGMDAAESERFLEGLVDW ACQAPRVHAHQWAAGDVVWVDNRCLLHRAEPWDFKLPRVMWHSRL AGRPETEGAALV ²⁹³
REFERENCE SUBSTANCES USED	1. Pre-stained molecular weight markers, Pierce Chemical Catalog #26691 (non-GLP) 2. Novagen Perfect Protein Markers, Catalog #69079 (non-GLP) 3. Bovine Serum Albumin (BSA), Pierce Chemical Catalog #23208 (non-GLP).

INITIATION DATE:28-Jan-2008**METHODS USED**PURITY/CONCENTRATION:SDS-PAGE & DensitometryQuantitative Amino Acid AnalysisIDENTIFICATION:SDS-PAGEWestern Blot

VERIFIED AS EXACT
COPY OF ORIGINAL
Initials BWS Date 19 MAR 2008

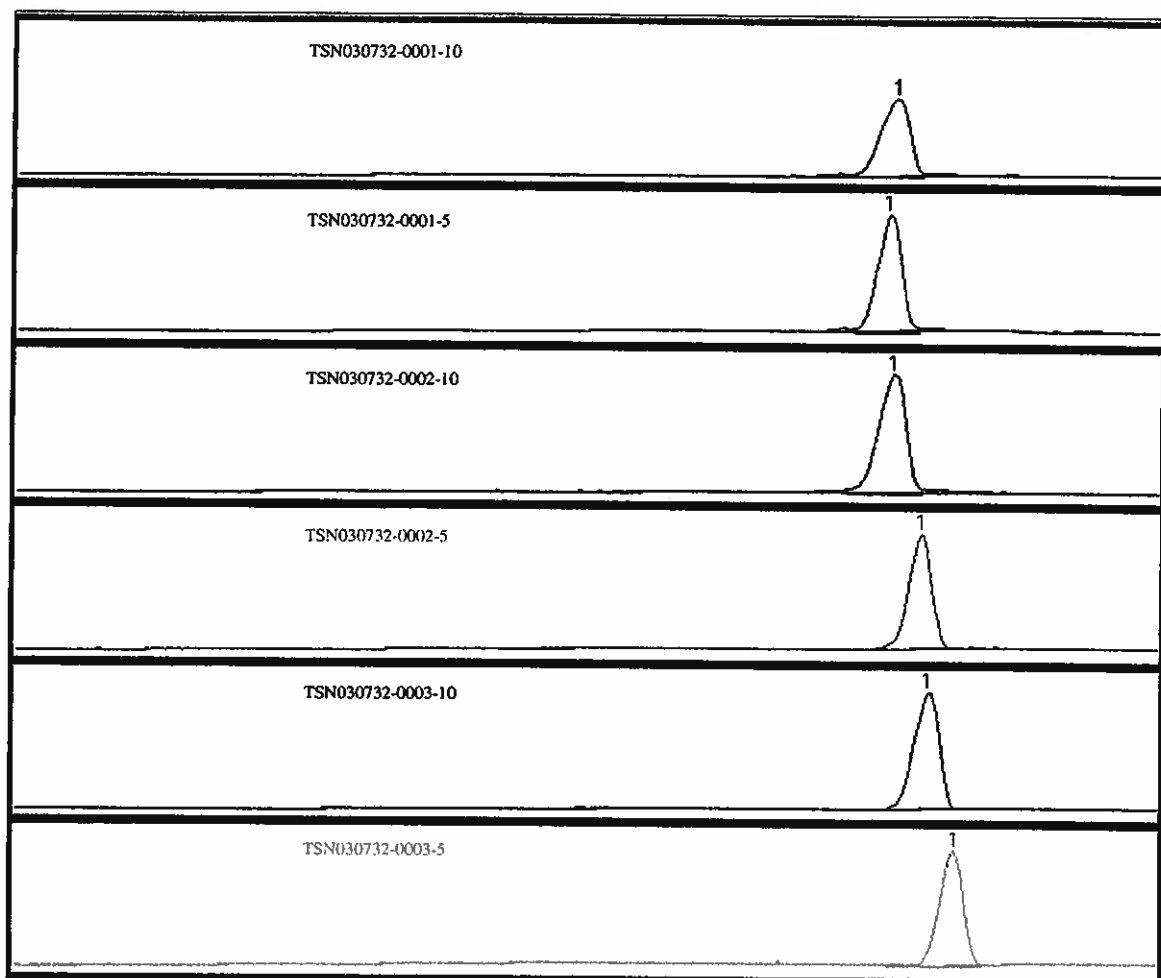
RESULTS AND CONCLUSIONS

X**IDENTITY****Figure 1: SDS-PAGE and Western blot of AAD-12 (TSN 030732)**

The apparent molecular weight of the truncated AAD-12 protein was as expected (~31.7 kDa).

Notes:

1. The proteins (resuspended at 1 mg/mL in 10 mM Tris, 100 mM NaCl, 20% glycerol, 0.1% Tween 20, pH 8.0) were separated with a 4 - 20% polyacrylamide gradient gel (Bio-Rad, Hercules, CA, Cat #: 161-1159) and the total protein was stained with GelCode Blue Protein Stain (Pierce, Rockford, IL, Cat #: 24592).
2. After the proteins were separated by SDS-PAGE, the proteins were transferred to a nitrocellulose membrane (Bio-Rad, Cat#: 162-0145), probed with anti-AAD-12 antibodies and developed with NBT/BCIP colorimetric detection (Sigma, St. Louis, MO, Cat #: B5655).
3. The Western blot confirmed the AAD-12 protein (TSN030732) was positive to a specific rabbit polyclonal antibody (Dow AgroSciences, lot # DAS1197-167-2) raised against the AAD-12 protein.
4. Due to the addition of colored dyes for visualization, the accuracy of the prestained (PS) molecular weight markers (Figure 1, left-hand side of the gel) are not as precise as the Novagen Perfect Protein Markers. The prestained markers are only used as an indication of protein transfer during blotting and molecular weight approximation.

Figure 2: Electrochromatograph of AAD-12 (TSN030732) Purity

Integration of the lanes containing the AAD-12 proteins (SDS-PAGE gel in Figure 1) detected only one protein peak. This peak correlates with the AAD-12 protein immunoreactive to antigen-specific antibodies (Dow AgroSciences, lot # F1197-167-2). Toxicological lots TSN030732-0001, -0002 and -0003 are >99% AAD-12 (protein/protein).

 X **INITIAL DETERMINATION**

The percentage of the AAD-12 protein in TSN030732-0001 powder is established by amino acid analysis to be 37.0%. The purity of the AAD-12 protein to total proteins in TSN030732-0001 is certified as >99%.

The percentage of the AAD-12 protein in TSN030732-0002 powder is established by amino acid analysis to be 35.3%. The purity of the AAD-12 protein to total proteins in TSN030732-0002 is certified as >99%.

The percentage of the AAD-12 protein in TSN030732-0003 powder is established by amino acid analysis to be 34.0%. The purity of the AAD-12 protein to total proteins in TSN030732-0003 is certified as >99%.

 NA **RECERTIFICATION: UNCHANGED**

Current value of _____ is within experimental variation of previously established purity of _____.
The purity is unchanged and remains as _____.

N.A. **RECERTIFICATION: RE-ESTABLISHED**

Current value of N.A. is NOT within experimental variation of previously
established purity of N.A. The purity is re-established as N.A.

N.A. **OTHER**

N.A.

RE-CERTIFICATION DUE DATE: April 1, 2009**CALCULATIONS:**

Four (4) aliquots of the lyophilized toxicology lots of the recombinant microbial AAD-12 (TSN030732-0001, -0002 and -0003) were accurately weighed and sent to the DAS ABCOE Laboratory for quantitative amino acid analysis and the results were compared to the recovery of a NIST certified BSA protein standard. The analysis was performed by personnel in the DAS ABCOE Small Molecule Group in a non-GLP setting. The results established the three individual tox lots to be 37.0, 35.3 and 34.0% AAD-12 respectively.

Table 1. Summary of AAD-12 Amino Acid Analysis Results

Sample	µg AAD-12/mg	Avg. Protein %RSD
TSN030732-0001	35.7	4.9
TSN030732-0001	35.6	5.0
TSN030732-0001	38.8	5.0
TSN030732-0001	37.9	5.1
Average	37.0	5.0
TSN030732-0002	34.9	4.7
TSN030732-0002	34.3	5.0
TSN030732-0002	36.3	4.7
TSN030732-0002	35.5	5.4
Average	35.3	5.0
TSN030732-0003	33.6	5.0
TSN030732-0003	33.4	5.7
TSN030732-0003	ND	ND
TSN030732-0003	35.0	4.8
Average	34.0	5.2


STUDY DIRECTOR SIGNATURE:


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AMENDED REPORT DATE:

19 MAR 2008

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18 Mar 2008

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TESTING FACILITY:

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All raw data associated with this study is recorded in the Dow AgroSciences archive. This study was conducted in accordance with the Good Laboratory Practice Standard, 40 CFR Part 160.135 (b) with the following exceptions. The GLP status of all commercial standards (protein molecular weight ladders, and bovine serum albumin from Novagen and Pierce) was unknown, and their chain of custody was not monitored. In addition, the quantitative amino acid analysis was performed in a non-GLP laboratory.