CERTIFICATE OF ANALYSIS OF THE TEST/REFERENCE/CONTROL SUBSTANCES: ARYLOXYALKANOATE DIOXYGENASE-12 (AAD-12) – TSN030732

TITLE/OBJECTIVE Recertification of the purity, concentration, and 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	**A**ryloxy**a**lkanoate **d**ioxygenase (abbreviation: AAD-12) expressed in recombinant *Pseudomonas fluorescens* strain DC579 (derived from host MB324 strain by transformation with pMYC1803 expression vector).
MOLECULAR WEIGHT	Approximately 31.6 kDa*
REFERENCE SUBSTANCES USED	1. Pre-stained molecular weight markers, Novex Sharp, Invitrogen, Catalog #LC5800 (non-GLP) 2. Unstained molecular weight markers, Novex Sharp, Invitrogen, Catalog #LC5801 (non-GLP) 3. Bovine Serum Albumin (BSA), Pierce Chemical Catalog #23208 (non-GLP) 4. BSA Standards, NIST SRM 927d (non-GLP) 5. Amino Acid Standard 10 pmol, Agilent Technologies, Catalog #5061-3334 (non-GLP) 6. Amino Acid Standard 25 pmol, Agilent Technologies, Catalog #5061-3333 (non-GLP) 7. Amino Acid Standard 100 pmol, Agilent Technologies, Catalog #5061-3332 (non-GLP) 8. Amino Acid Standard 250 pmol, Agilent Technologies, Catalog #5061-3331 (non-GLP) 9. Amino Acid Standard 1000 pmol, Agilent Technologies, Catalog #5061-3330 (non-GLP)

***Note: The N-terminal methionine of the AAD-12 protein was removed by an aminopeptidase**

INITIATION DATE: January 4, 2011

Table 1:METHODS USED

Characteristics	Methods Used	TSN030732-0001 Results	TSN030732-0002 Results	TSN030732-0003 Results
Concentration	Amino Acid Analysis	34.5%	33.1%	33.7%
Purity	SDS-Page/Densitometry	>99%	>99%	>99%
Molecular Weight	SDS-Page/Densimotetry	~32 kDa	~32 kDa	~32 kDa
Identity	Western Blot	Confirmed	Confirmed	Confirmed
Activity	Enzymatic Assay	Confirmed	Confirmed	Confirmed

X **RECERTIFICATION: UNCHANGED**

The current value of 34.5% of AAD-12 protein in TSN030732-0001 is within experimental variation of previously established concentration of 37.0% (370 µg/mg) . **The concentration is unchanged and remains as 37.0% .** .

The current value of 33.1% of AAD-12 protein in TSN030732-0002 is within experimental variation of previously established concentration of 35.3% (353 µg/mg). **The concentration is unchanged and remains as 35.3%.**

The current value of 33.7% of AAD-12 protein in TSN030732-0003 is within experimental variation of previously established concentration of 34.0% (340 µg/mg). **The concentration is unchanged and remains as 34.0%.**

X **CALCULATIONS**

Three (3) aliquots of the lyophilized toxicology lots of the recombinant microbial AAD-12 (TSN030732-0001, TSN030732-0002, and TSN030732-0003) were weighed on an analytical balance. The samples were analyzed by quantitative amino acid analysis (***each sample was run in triplicate***) and the results were compared to the recovery of a NIST BSA protein standard. The results re-established the AAD-12 proteins to be **370µg/mg** (TSN030732-0001), **353µg/mg** (TSN030732-0002).and **340ug/mg** (TSN030732-0003). See results in Table 2.

Table 2. Summary of AAD-12 Amino Acid Analysis Results

Sample Identification	µg AAD-12/mg	Concentration (w/w) (%)
AAD-12 (TSN030732-0001-1)	352	35.2
AAD-12 (TSN030732-0001-2)	343	34.3
AAD-12 (TSN030732-0001-3)	339	33.9
Average	**344.7**	34.5
StdDev.	-	0.7

Sample Identification	µg AAD-12/mg	Concentration (w/w) (%)
AAD-12 (TSN030732-0002-1)	327	32.7
AAD-12 (TSN030732-0002-2)	335	33.5
AAD-12 (TSN030732-0002-3)	331	33.1
Average	**331**	33.1
StdDev.	-	0.4

Sample Identification	µg AAD-12/mg	Concentration (w/w) (%)
AAD-12 (TSN030732-0003-1)	333	33.3
AAD-12 (TSN030732-0003-2)	340	34.0
AAD-12 (TSN030732-0003-3)	338	33.8
Average	**337**	33.7
StdDev.	-	0.4

RE-CERTIFICATION DUE DATE: April 1, 2015

STUDY DIRECTOR SIGNATURE:

Shawna Embrey

STUDY COMPLETION DATE: 01 Apr 2011

PEER REVIEWER SIGNATURE:

Nick Harpham

DATE: 31-Mar-2011

PEER REVIEWER SIGNATURE:

DATE:

Barry Schafer

31-MAR-2011

TESTING FACILITY:

Regulatory Sciences and Government Affairs
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All raw data associated with this study will be archived 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 markers, amino acid standards and bovine serum albumin from Invitrogen, Thermo-Pierce, NIST and Agilent Technologies, respectively) was unknown, and their chain of custody was not monitored.