

**CERTIFICATE OF ANALYSIS OF THE TEST/REFERENCE/CONTROL SUBSTANCE:
2mEPSPS PROTEIN (TSN033171-0001)**

TITLE/OBJECTIVE Certification of the purity, concentration, and identity of the following test/reference/control substance for use in a study.

TEST/REFERENCE/CONTROL SUBSTANCE

LOT	TSN033171-0001
DESCRIPTION	2mEPSPS purified from recombinant <i>Pseudomonas fluorescens</i> DC454 Lot #: DMMG 033110
MOLECULAR WEIGHT	Approximately 47.3 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. wtEPSPS, microbe-derived, concentration (~23% a.i.) – TSN032933-0002 (non-GLP)

INITIATION DATE:

April 16, 2010

METHODS USED

Characteristics	Methods Used	Results
Concentration	SDS-PAGE/Densitometry	665 µg a.i./mg powder
Purity	SDS-PAGE/Densitometry	>99% pure
Molecular Weight	Mass Spectrometry	47.3 kDa
Identity	Mass Spectrometry	Complete AA sequence confirmed
Identity	Western Blot	Confirmed
Activity	Enzyme Assay	Confirmed

X

INITIAL DETERMINATION

The amount of 2mEPSPS contained in the lyophilized powder is established by SDS-PAGE/densitometry as 665 µg/mg. The standard deviation was 10.2%.

The purity of the sample (protein/protein) is established by SDS-PAGE/densitometry as >99% pure.

VERIFIED AS EXACT
COPY OF ORIGINAL

Initials BWS Date 01 OCT 2010

X **OTHER**

The enzymatic assay performed was a modified version of Forlani, G., *et al.*, 5-enol-Pyruvyl-Skikimate-3-phosphate Synthase from *Zea mays* Cultured Cells, Plant Physiol. (1994) 105: 1107-1114

X **CALCULATIONS**

Analyst	Sample Identification	2mEPSPS Concentration (µg/mg)
Analyst 1	Replicate 1	659
	Replicate 2	671
	Average	665
	Stdev	10.2%

For SDS-PAGE/densitometry analysis, a sample of TSN033171-0001 was diluted in phosphate buffered saline with Tween 20 (pH 7.4, Sigma catalog #P-3813) and separated on a pre-cast Criterion gel (Bio-Rad, catalog #:345-0123). A standard curve was generated using absorbance readings from dilutions of wild-type EPSPS (TSN032933-0002) via ImageQuant software (Molecular Dynamics, S/N #56680). The final concentration determination was the average of 2 replicates and was independently confirmed by Karnoup and Kuppannan at The Dow Chemical Company.

RE-CERTIFICATION DUE DATE:

October 2011


STUDY DIRECTOR SIGNATURE:


Barry W. Schafer

STUDY COMPLETION DATE:

01-Oct-2010

PEER REVIEWER SIGNATURE:


Shawna K. Embrey

DATE:

01 Oct 2010

TESTING FACILITY:

Regulatory Sciences and Government Affairs
Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, Indiana 46268

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 and bovine serum albumin from Invitrogen and Pierce) was unknown, and their chain of custody was not monitored. The mass spectrometry characterization of the protein was conducted in a non-GLP laboratory.