

SAGE Accession Number



272135

## FAPC REPORT

### Attention!

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Item ID: GLP027988  
Local Call Number: GLP027988  
  
FAPC/BIOT Protocol Number: BIOT09-203839

Study ID:  
Contract Lab:  
Authors: Schafer, Barry W.  
Date Completed: 20090505  
Compound: Phosphinothricin Acetyltransferase (PAT)  
Test Substance Number: TSN031116-0001  
Lot Number: 55238-1A  
Related Accession Number: 279438

Item ID



GLP027988

1 GLP Archive

**CERTIFICATE OF ANALYSIS OF THE TEST/REFERENCE/CONTROL SUBSTANCE:  
PHOSPHINOTHRICIN ACETYLTRANSFERASE (PAT - TSN031116-0001)**

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

**TEST/REFERENCE/CONTROL SUBSTANCE**

LOT	55238-1A
DESCRIPTION	Microbe-derived PAT (phosphinothricin acetyltransferase) protein. Isolated from recombinant <i>E. coli</i> . Liquid preparation.
MOLECULAR WEIGHT	Approximately 20.6 kDa
PROTEIN SEQUENCE OF PAT	<div>1 MSPERRPVEIRPATAADMAAVCDIVNHYIE 30</div> <div>31 TSTVNFRTPEPTQEWIDDLERLQDRYPWL 60</div> <div>61 VAEVEGVVAGIAYAGPWKARNAYDWTVEST 90</div> <div>91 VYVSHRHQRLGLGSTLYTHLLKSMEAQGFK 120</div> <div>121 SVVAVIGLPNDPSVRLHEALGYTARGTLRA 150</div> <div>151 AGYKHGGWHDVGFWRQDFELPAPPRPVRPV 180</div> <div>181 TQI 183</div>
REFERENCE SUBSTANCES USED	<div>1. Pre-stained molecular weight markers, Novex Sharp, Invitrogen, Catalog #LC5800 (non-GLP)</div> <div>2. Unstained molecular weight markers, Mark12, Invitrogen, Catalog #LC5677 (non-GLP)</div> <div>3. Bovine Serum Albumin (BSA), Pierce Chemical Catalog #23208 (non-GLP).</div> <div>4. PAT, Dow AgroSciences TSN105742, 0.30 mg/mL</div>

**INITIATION DATE:**

January 30, 2009

**METHODS USED****PURITY/CONCENTRATION:**

ELISA

Densitometry

**IDENTIFICATION:**

SDS-PAGE

Western Blot

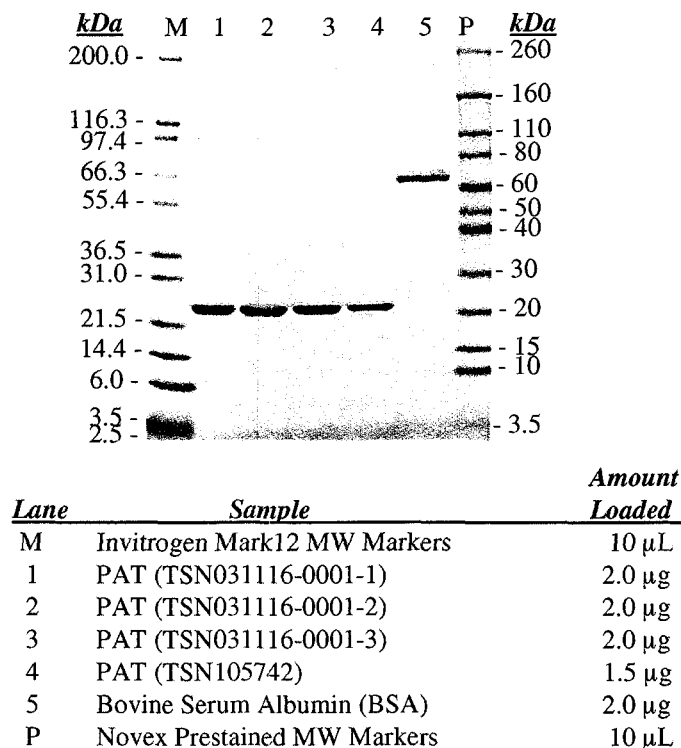
Enzymatic activity assay

## RESULTS

X

## IDENTITY

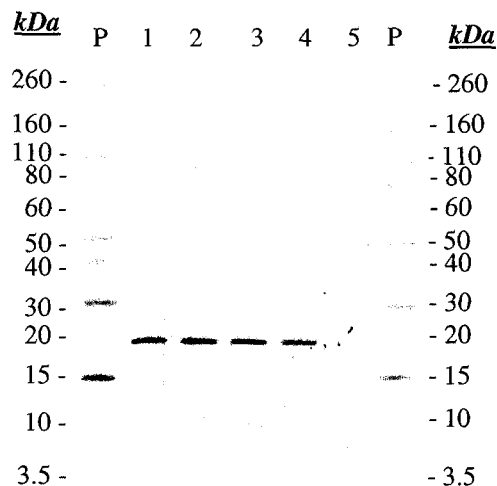
Figure 1: SDS-PAGE of PAT (TSN031116-0001)



The apparent molecular weight of the PAT in TSN031116-0001 was as expected (~20kDa), which is the same as the reference standard TSN105742.

1. For SDS-PAGE the proteins were separated with a 4 - 20% polyacrylamide gradient gel (Bio-Rad, Hercules, CA, Cat #: 345-0032) and the total protein was stained with GelCode Blue Protein Stain (Pierce, Rockford, IL, Cat #: 24592).
2. The unstained molecular weight standards demonstrated a slight mobility change when electrophoresed in the alkaline SDS-PAGE buffer system (pH = 8.3). The pH of the SDS-PAGE buffer can affect the charge of proteins and their binding capacity for SDS (which affects the protein mobility). Therefore the test protein (PAT TSN031116-0001) and the reference protein (PAT TSN105742) reflect a similar migration pattern that appears to have a higher apparent molecular weight.

X

**IDENTITY CON'T****Figure 2: Western blot of PAT (TSN031116-0001)**

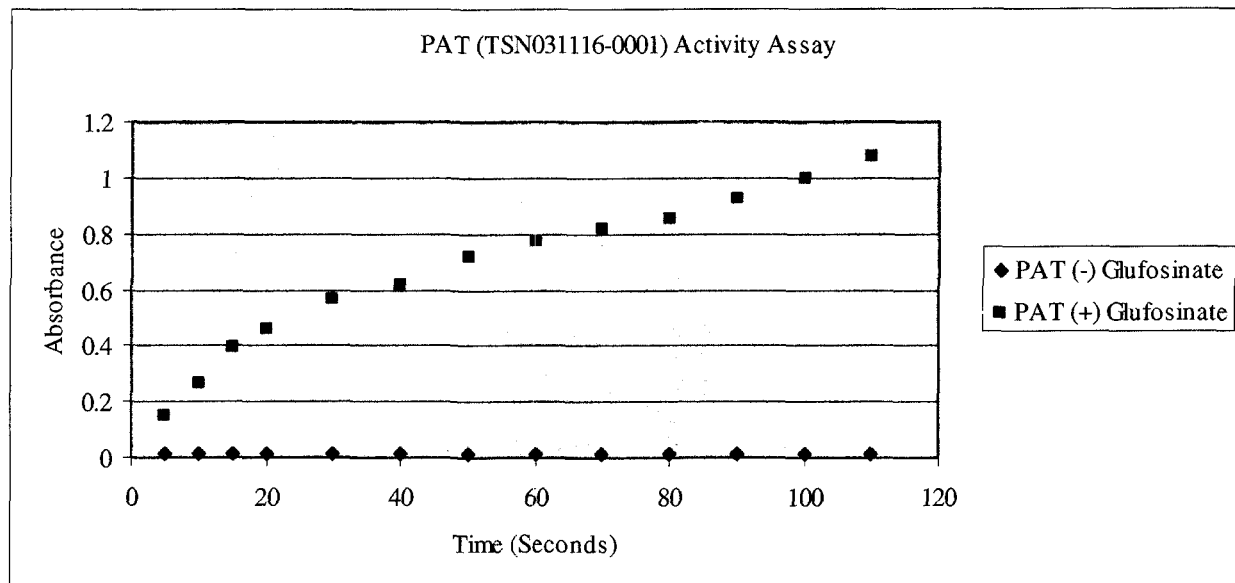
<i>Lane</i>	<i>Sample</i>	<i>Amount Loaded</i>
P	Novex Prestained MW Markers	10 $\mu$ L
1	PAT (TSN031116-0001-1)	35 ng
2	PAT (TSN031116-0001-2)	35 ng
3	PAT (TSN031116-0001-3)	35 ng
4	PAT (TSN102172)	27 ng
5	Bovine Serum Albumin (BSA)	37.5 ng
P	Novex Prestained MW Markers	10 $\mu$ L

**Notes:**

1. The liquid tox lot TSN031116-0001 was diluted in PBST (Sigma, St. Louis, MO, Cat#: P3563) buffer.
2. For the western blot, the proteins were separated with a 4-12% gradient gel (Bio-Rad, Hercules, CA, Cat #: 345-0123). After separation the proteins were transferred to a nitrocellulose membrane (Bio-Rad, Cat#: 162-0213), probed with anti-PAT antibodies and developed with colorimetric detection (SigmaFast NBT/BCIP, Cat #: B5655).
3. The western blot confirmed the mouse monoclonal antibody (DAS 155AD-4) raised against the PAT protein were specific to the PAT proteins present in the tox lot TSN031116-0001.

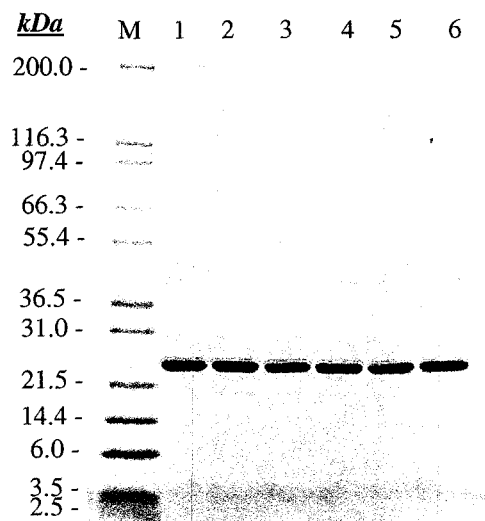
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IDENTITY CON'T

**Figure 3: Absorbance plot of PAT (TSN1031116-0001) activity**

Using a Shimadzu UV160U, UV-visible spectrophotometer, a reaction cocktail containing PAT (TSN1031116-0001) was measured over time to determine activity. In Figure 3 absorbance readings of PAT in the presence of Glufosinate shows the catalyzation reaction between the Glufosinate and acetyl-CoA. The absence of Glufosinate (negative control) detects no change in the reaction mixture.

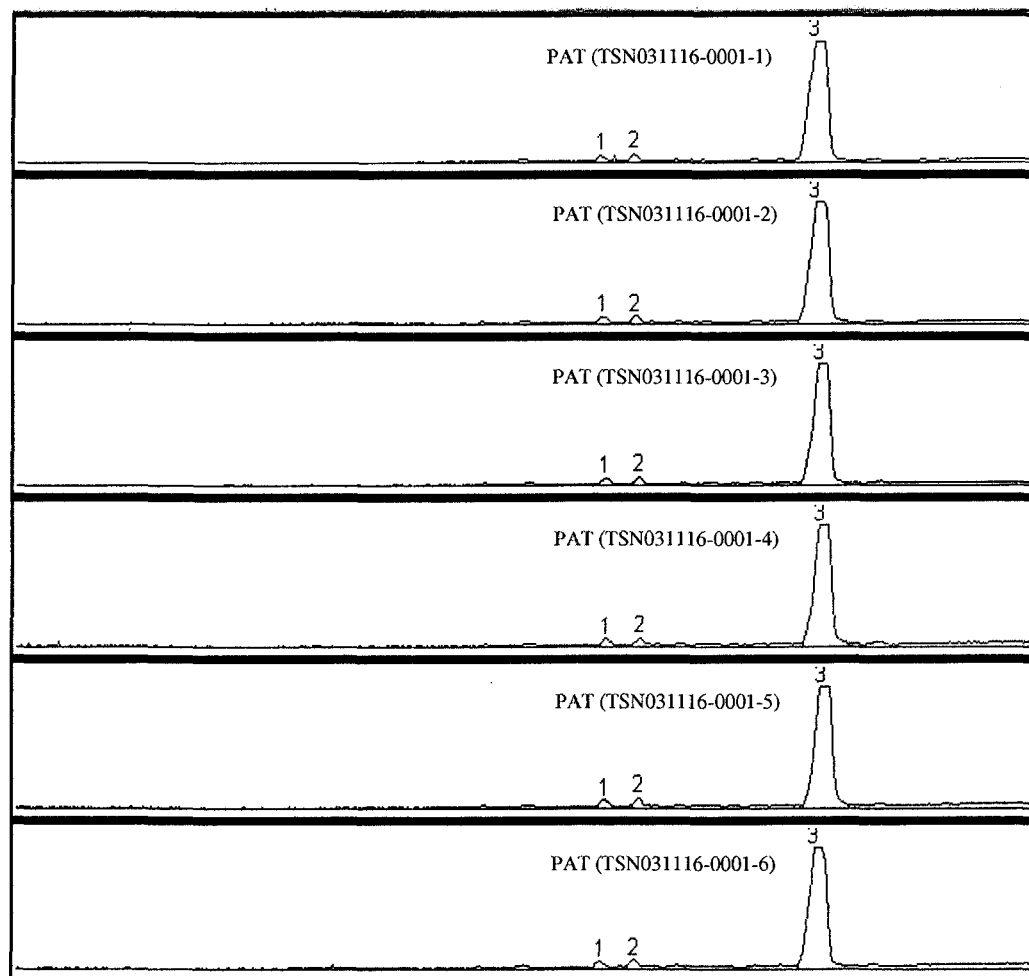
X

**PURITY/CONCENTRATION****Figure 4: Densitometer scanned SDS-PAGE (4-12% gradient) image**

Lane	Sample	Amount
M	Marker	10 µL
1	PAT (TSN031116-0001-1)	2.0 µg
2	PAT (TSN031116-0001-2)	2.0 µg
3	PAT (TSN031116-0001-3)	2.0 µg
4	PAT (TSN031116-0001-4)	2.0 µg
5	PAT (TSN031116-0001-5)	2.0 µg
6	PAT (TSN031116-0001-6)	2.0 µg

After staining, the gel was scanned with the Personal Densitometer SI (Molecular Devices, Sunnyvale, California) using ImageQuant Scanner Control software. The image was used to calculate the percentage of PAT protein in the preparation.

X

**PURITY/CONCENTRATION CON'T****Figure 5. Electrochromatograph Determination of PAT Protein (TSN031116-0001) Purity**

Peak 3 in Figure 5 is the PAT protein. The PAT protein to the total protein in the toxicology lot was determined as 94% (average of six measurements) using ImageQuant software.

X**INITIAL DETERMINATION**

The concentration of the PAT protein in solution is established as 0.81 mg/mL. The standard deviation was +/- 0.02 mg/mL.

The PAT protein purity in terms of its percentage to the total proteins (PAT plus other proteins) was determined as 94% (Figure 5).

Note 1: The PAT (TSN031116-0001) standard was reconstituted in the following buffer; 50% Glycerol, 100 mM Tris, 500 mM NaCl, pH 7.5.

NA**RECERTIFICATION: UNCHANGED**

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

NA**RECERTIFICATION: RE-ESTABLISHED**

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

N.A.**OTHER**

N.A.

**RE-CERTIFICATION DUE  
DATE:**

March 30, 2014



<b>CALCULATIONS</b>
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Six (6) aliquots (designated as replicate #1, #2, #3, #4, #5, #6) of the liquid toxicology lot of the recombinant microbial PAT (TSN031116-0001) were diluted in phosphate buffered saline with Tween 20 (pH 7.4, Sigma catalog #P-3813). For ELISA (enzyme linked immunosorbent assay), the solutions were diluted 30000 times in PBST and distributed to 4 analysts. Each analyst diluted the samples 1:5 with PBST and performed ELISA on each of the samples independently. In each assay, standard curves were generated using quadratic function fits of the absorbance readings from the standard PAT (TSN105742) via SOFTmax®Pro software (version 4.0, Molecular Devices Corporation, Sunnyvale, California). Serial dilutions were performed on replicates of TSN031116-0001 to determine the appropriate dilutions within the linear range of the standard curves. Dilutions with readings within the range of standard curve were selected to calculate the PAT protein concentration. The final purity data was the average of 24 measures (6 aliquots x 4 analysts).

**Table 1. PAT ELISA results**


Sample Identification	Analyst 1	Analyst 2	Analyst 3	Analyst 4	Mean
TSN031116-0001-1	0.90	0.82	0.83	0.76	0.83
TSN031116-0001-2	0.84	0.77	0.78	0.71	0.77
TSN031116-0001-3	0.91	0.81	0.83	0.72	0.82
TSN031116-0001-4	0.87	0.88	0.85	0.72	0.83
TSN031116-0001-5	0.89	0.87	0.87	0.74	0.84
TSN031116-0001-6	0.86	0.83	0.83	0.70	0.80
Grand Mean					0.81
Stdev					0.02

*Values in the table above were calculated before rounding.*

**REPORT**

Study ID: BIOT09-203839

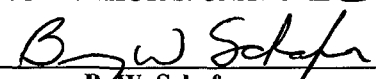
**STUDY DIRECTOR SIGNATURE:**

  
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S. K. Embrey

**STUDY COMPLETION DATE:**

5-May-2009

**CO-AUTHOR SIGNATURE**

  
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B. W. Schafer

**DATE:**

5-may. 2009

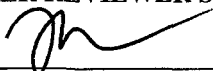
**PEER REVIEWER SIGNATURE:**

  
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**DATE:**

5-May, 2009

**PEER REVIEWER SIGNATURE:**

  
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R. A. Herman

**DATE:**

5-May-2009

**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.