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Western Blot Analysis of Cry1Ab and PAT Proteins Expressed in Field  
Corn

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Report No. SSB-112-05

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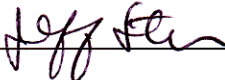
## STATEMENT OF DATA CONFIDENTIALITY CLAIMS

Claims of confidentiality are made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d) (1) (A), (B), or (C).

Company: Syngenta Seeds, Inc. – Field Crops - NAFTA

Company Agent: Jeff Stein Date: \_\_\_\_\_

Title: Head of NAFTA Biotech Regulatory Affairs

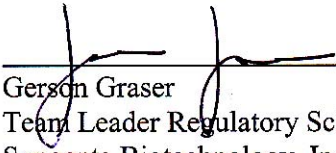
Signature: \_\_\_\_\_  


These data are the property of Syngenta Seeds, Inc. – Field Crops – NAFTA and, as such, are considered to be confidential for all purposes other than compliance with FIFRA §10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality that may exist under any other statute or in any other country.

**STATEMENT CONCERNING GOOD LABORATORY PRACTICES**


This study was not conducted in compliance with the relevant provisions of Good Laboratory Practice Standards (40 CFR 160, Federal Register, 1989) pursuant to the Federal Insecticide, Fungicide and Rodenticide Act, and subsequent revisions.

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**SYNGENTA BIOTECHNOLOGY, INC.  
REGULATORY SCIENCE & PRODUCT SUPPORT  
RESEARCH TRIANGLE PARK, NC, USA**

**REPORT NO. SSB-112-05**

**Western Blot Analysis of Cry1Ab and PAT Proteins Expressed in Field Corn**

***SUMMARY***

Western blot analyses of leaf extracts of Event Bt-11 and Event Bt-10-derived corn plants revealed similar dominant immunoreactive bands in both events, corresponding to the predicted Cry1Ab protein (Cry1Ab) and phosphinothricin acetyltransferase (PAT) molecular weight of *ca.* 69,000 and 22,000 daltons, respectively.

***INTRODUCTION***

The purpose of this study was to examine the size and immunoreactivity of the Cry1Ab and PAT expressed in maize plants derived from the field corn Events designated Bt-11 and Bt-10. In this study Cry1Ab and PAT proteins were identified by Western blot analysis after extracting both proteins from leaf tissue.

## ***MATERIALS AND METHODS***

**Preparation of Cry1Ab Plant Extracts.** Leaf tissue (ca. 0.5g) derived from Bt-11- and Bt-10-derived maize plants were ground in a mortar with liquid nitrogen and subsequently suspended in 1.5 ml extraction buffer A, which consisted of 50mM CAPS (3-cyclohexylamino-1-propanesulfonic acid) containing 100 mM NaCl, 2mM EDTA (ethylenediaminetetraacetate), 2 mM DTT (dithiothreitol), 20 µl/ml protease inhibitor cocktail (Complete, Roche, 1 tablet/ml) in accordance with Standard Operating Procedure (SOP) 2.7. The mixture was extracted for 30 min on ice and then centrifuged for 10 min at 15,000 x g. The supernatant was directly used for Western blot analysis.

**Preparation of PAT Plant Extracts.** Leaf tissue (ca. 40 mg) derived from Bt-11- and Bt-10-derived maize plants were macerated with a pestle (Kontes, Vineland, NJ, USA) in a 1.5 ml standard reaction tube, subsequently suspended in 0.2 ml extraction buffer A (as described above) and centrifuged for 3 min at 15,000 x g. The supernatant was directly used for Western blot analysis.

**Immunoreactivity and Molecular Weight Determination.** Samples of the leaf extracts of Bt-11 and Bt-10 as described above were subjected to SDS-PAGE followed by electroblotting (Western blot analysis) in accordance with SOP 2.3 and 2.4. Dilutions prepared in SDS sample buffer containing *ca.* 30 ng Cry1Ab and *ca.* 15 ng PAT, as semi-quantitatively determined by comparing band intensities of characterized Cry1Ab and PAT standards with the intensities of the bands detected for the test proteins (data not shown). SeeBlue<sup>®</sup> Plus molecular weight standard (15 µl, Invitrogen; San Diego, CA, USA) and Kaleidoscope Precision Plus Protein Standard (8 µl, BioRad, Hercules, CA, USA) was used to establish approximate molecular weights for Cry1Ab and PAT, respectively. After electroblotting, the membrane was probed with immunoaffinity-purified rabbit anti-Cry1Ab polyclonal antibodies for Cry1Ab and with immunoaffinity-purified rabbit anti-PAT polyclonal antibodies for PAT. Donkey anti-rabbit IgG linked to alkaline phosphatase (Jackson; West Grove, PA, USA), diluted (1:3,000 for Cry1Ab and 1:10,000 for PAT) in blocking buffer, was used to bind to the primary antibody and was visualized by development with alkaline phosphatase substrate solution. The Western blots were examined for the presence of intact immunoreactive Cry1Ab (*ca.* 69,000 molecular weight), PAT (*ca.* 22,000 molecular weight) and other immunoreactive Cry1Ab or PAT polypeptides.

## ***RESULTS***

**Immunoreactivity and Molecular Weight Determination.** Western blot analysis of the Cry1Ab in plant leaf extracts of Bt-11 and Bt-10 revealed a dominant immunoreactive band corresponding to the predicted molecular weight of Cry1Ab (*ca.* 69,000 Da; Figure 1). In both samples two minor breakdown fragments at *ca.* 52 and 46 kDa were evident. Western blot analysis of the PAT in plant leaf extracts of Bt-11 and Bt-10 revealed a dominant immunoreactive band corresponding to the predicted molecular weight of PAT (*ca.* 22,000 Da; Figure 2).

## ***CONCLUSIONS***

Cry1Ab and PAT proteins from plant leaf extracts of Bt-11 and Bt-10 were determined to have the predicted molecular weight of *ca.* 69,000 Da and 22,000 Da, respectively, and further immunologically cross-reacted with the corresponding (anti-Cry1Ab or anti-PAT) antibody.

There were no circumstances that may have affected the quality or integrity of the data generated in this study.

## ***RECORDS RETENTION***

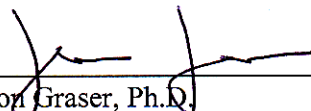
The original copy of this report are archived at Syngenta Biotechnology, Inc., 3054 Cornwallis Rd., Research Triangle Park, NC, USA 27709.

## ***STATISTICAL METHODS***

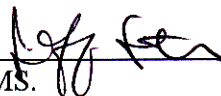
Statistical analyses were not required in this study.

## ***CONTRIBUTING SCIENTISTS***

Analytical work reported herein was conducted by Xinlan Li, M.S., Gerson Graser, Ph.D. and Michelle Yarnall, M.S.

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Feb 11, 2005  
Date

Approved by:   
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Director of  
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Feb 11, 2005  
Date

## ***REFERENCES***

### **Standard Operating Procedures**

SOP 2.3	Western Blot Analysis
SOP 2.4	SDS-Polyacrylamide Gel Electrophoresis
SOP 2.7	Cry1Ab Extraction From Maize Tissue and Silage

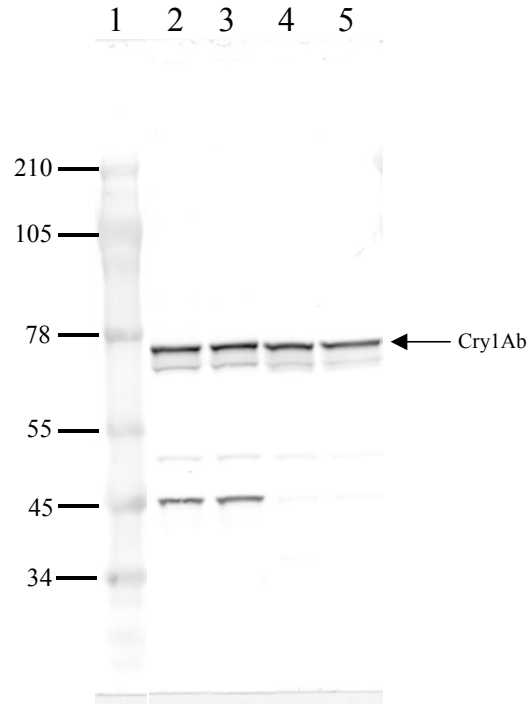
**Figure 1. Western Blot Analysis of Leaf Extracts of Event Bt-11 and Bt10 Using Cry1Ab-Specific Antibodies**

Lane 1: Molecular weight marker SeeBlue Plus2 (Invitrogen)

Lanes 2 and 3: protein extract from Event Bt-11 leaf tissue

Lanes 4 and 5: protein extract from Event Bt-10 leaf tissue

The molecular weight of the Cry1Ab protein corresponds to ca. 68,900 Da (see arrow)



**Figure 2. Western Blot Analysis of Leaf Extracts of Event Bt-11 and Bt-10 Using PAT-Specific Antibodies**

Western blot analysis:

Lane 1 – MW markers (BioRad, Kaleidoscope)

Lane 2 – purified PAT protein (standard)

Lane 3 – protein extract from Event Bt-10 leaf tissue

Lane 4 – protein extract from Event Bt-11 leaf tissue

The molecular weight of the PAT protein corresponds to ca. 22,000 Da (see arrow)

