Study Title

Assessment of the Equivalence of CP4 EPSPS Protein Expressed in *Escherichia coli* and in Roundup Ready® Corn Lines NK600 and NK603

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Performing Laboratory

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Statement of No Data Confidentiality Claims

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10(d)(l)(A), (B), or (C).

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Company: Monsanto Company

Company Agent: Kent Croon, Ph.D

Title: Regulatory Affairs Manager

Signature: _____ Date: 9/30/99

Statement of Compliance

This study meets the GLP requirements for 40 CFR Part 160 (EPA).

Submitter: _____ Date: 9/30/99

Sponsor: Ravinder S Sidhu Date: 9/30/99

Study Director: _____ Date: 9/30/99

Quality Assurance Statement

Reviews conducted by the Quality Assurance Unit confirm that the final report reflects the raw data.

Following is a list of reviews conducted by the Monsanto AG Regulatory QAU on the study reported herein.

Dates of Inspection/Audit	**Phase**	**Study Director**	**Date Reported To: Management**
09/14/1999	Western Blot	09/22/1999	09/22/1999
09/28/1999	Final Report and Data	09/30/99	09/30/99

Barbara Foy
Quality Assurance Specialist
Monsanto Company

9/30/99
Date

Signatures of Approval

Study Number:	99-01-46-50
Title:	Assessment of the Equivalence of CP4 EPSPS Protein Expressed in *Escherichia coli* and in Roundup Ready® Corn Lines NK600 and NK603
Facilities:	Monsanto Company Biotechnology Regulatory Sciences 700 Chesterfield Parkway North St. Louis, Missouri 63198, USA
Sponsor:	Ravinder Sidhu, Ph.D.
Study Director:	Thomas C. Lee, Ph. D.
Study Initiation Date:	September 10, 1999
Study Completion Date:	September 30, 1999
Records Retention:	All study specific raw data, final reports and facility records will be retained at Monsanto, St. Louis.

Signatures of Approval:

Ravinder S Sidhu — 9/30/99

Sponsor — Date

Thomas C. Lee — 9/30/99

Study Director — Date

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Abbreviations

~	Approximately
°C	Degree Celsius
CaMV	Cauliflower mosaic virus
CFR	Code Federal Regulations
CP4 EPSPS	5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4
CTP	Chloroplast transit peptide
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
EPA	Environmental Protection Agency
FIFRA	Federal Insecticide Fungicide Rodenticide Act
GLP	Good Laboratory Practices
g, mg, µg	Gram, milligram, microgram
HRP	Horse radish peroxidase
min	Minute(s)
M, mM, µM	Molar, millimolar, micromolar
mL, µL or µl	Milliliter, microliter
MW	Molecular weight
NaCl	Sodium chloride
NFDM	Nonfat dried milk
NOS	Nopaline synthase
PCR	Polymerase Chain Reaction
PMSF	Phenylmethylsulfonyl fluoride
PVPP	Polyvinylpolypyrrolidone
rpm	Revolutions per minute
RR	Roundup Ready®
SDS	Sodium dodecylsulfate
SDS-PAGE	Sodium dodecylsulfate-polyacrylamide gel electrophoresis
SOP	Standard Operating Procedure
Tris-Cl	Tris(hydroxymethyl)aminomethane as the chloride salt
TBST	Tris buffered saline with Tween
Tween 20	Polyoxyethylenesorbitan mono laurate
(v/v)	Volume to volume ratio
(w/v)	Weight to volume ratio

I. SUMMARY

Monsanto Company has developed Roundup Ready® corn lines NK600 and NK603 which are tolerant to glyphosate (the active ingredient in Roundup® herbicide) at the whole plant level. These corn lines contain a 5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium* sp. strain CP4 (CP4 EPSPS). Corn plants that demonstrate commercial level of tolerance to Roundup herbicide are called Roundup Ready® (RR). The CP4 EPSPS gene from *Agrobacterium* sp. strain CP4 has been completely sequenced and encodes a 47.6-kDa protein consisting of a single polypeptide of 455 amino acids (Padgette *et al.*, 1996). The CP4 EPSPS protein is functionally similar to plant EPSPS enzymes but has a much reduced affinity for glyphosate (Padgette *et al.*, 1993). In nontransgenic plants, glyphosate binds to the plant EPSPS enzyme and blocks the biosynthesis of aromatic amino acids thereby starving plants of these essential nutrients (Steinrucken and Amrhein, 1980; Haslam, 1993). In Roundup Ready® plants, nutritional requirements for normal growth and development are met by the continued action of the glyphosate-tolerant CP4 EPSPS enzyme in the presence of glyphosate. A comprehensive safety assessment of the CP4 EPSPS protein has been described in the literature (Harrison *et al.*, 1996).

NK603 is the line for which regulatory approval is currently requested; NK600 is a backup line and was included in this study as additional information for comparison purposes. Corn line NK603 was produced by transformation of corn tissue with a 6.7-kb linear DNA fragment PV-ZMGT32L derived from the plasmid vector PV-ZMGT32, using a particle acceleration method. Molecular analysis (Deng *et al.*, 1999) has shown that corn line NK603 contains a single DNA insert consisting of two expression cassettes: the first CP4 EPSPS gene cassette, containing the CP4 EPSPS coding sequence under regulation of the rice actin promoter and intron (P-ract1/ract intron), a chloroplast transit peptide (CTP2) sequence, and a nopaline synthase (NOS) 3' polyadenylation sequence; and second CP4 EPSPS gene cassette, containing the CP4 EPSPS coding sequence under the regulation of the cauliflower mosaic virus (CaMV) enhanced 35S plant promoter (e35S), a maize heat-shock protein 70 (*Zm*HSP70) intron, CTP2, and the NOS 3' polyadenylation sequence.

The purpose of this study was to assess the equivalence of 5-enol-pyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4 (CP4 EPSPS) produced in *Escherichia coli* (*E. coli*) and in the two Roundup Ready® corn (*Zea mays* L.) lines NK600 and NK603 using the Western blot analytical methodology (immunoblotting). Immunoblotting is a highly specific and sensitive method for the comparison of apparent molecular weights and immunological properties of low abundance proteins contained in complex mixtures such as grain extracts. A protein extract prepared from a commercial variety of RR soybeans (AG3701) was included in this study for comparison. Regulatory approval for RR soybean was obtained in the U.S. in 1995 and is currently grown on approximately 40 million acres. CP4 EPSPS protein was purified from RR soybeans and an extensive characterization study

was conducted that demonstrated its equivalence to the *E. coli*-expressed standard (lot # 5192245, Harrison *et al.*, 1993a). This current study also serves to characterize these Roundup Ready® corn events with respect to the CP4 EPSPS protein. This study will be part of the data submitted to regulatory agencies supporting the commercialization of Roundup Ready® corn plants containing the CP4 EPSPS protein.

CP4 EPSPS protein produced in Roundup Ready® corn lines NK600 and NK603 was demonstrated to be equivalent to both the CP4 EPSPS protein expressed in and purified from *E. coli* for safety studies and CP4 EPSPS expressed by a commercial RR soybean variety. Equivalence was based on visually identical apparent molecular weights (~47 kD) and immunological properties when detected using antibodies specific for CP4 EPSPS protein. CP4 EPSPS is expressed a an N-terminal fusion with a chloroplast transit peptide (CTP2). These results provide indirect evidence that the chloroplast transit peptide (CTP2) is properly processed upon chloroplast import yielding the "mature" CP4 EPSPS of the same size as that expressed in RR soybeans and the *E. coli*-expressed protein used for safety studies.

In conclusion, this demonstration of equivalence justifies the application of the safety data generated using the *E. coli* produced CP4 EPSPS protein for the CP4 EPSPS protein produced in Roundup Ready® corn lines NK600 and NK603.

II. INTRODUCTION

A. Background. Monsanto Company has developed Roundup Ready® corn lines NK600 and NK603 which are tolerant to glyphosate (the active ingredient in Roundup™ herbicide) at the whole plant level. These corn lines express a glyphosate tolerant 5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium* sp. strain CP4 (CP4 EPSPS). Other Roundup Ready® crops such as RR soybeans, RR canola, RR sugarbeets and RR cotton have also been developed by Monsanto. Tolerance to glyphosate for these RR crops is also based on the endogenous expression of CP4 EPSPS. A comprehensive safety assessment of the CP4 EPSPS protein has been described in the literature (Harrison *et al.*, 1996). Because the level of expression of CP4 EPSPS is low in RR crops (including RR corn lines NK600 and NK603) it was not feasible to isolate sufficient CP4 EPSPS from these plants to conduct all of the safety studies. Therefore, the safety of the CP4 EPSPS protein was assessed using CP4 EPSPS protein expressed in and purified from *E. coli* (lot # 5192245). Studies have been conducted that verify the physical and functional equivalence of the plant- and *E. coli* (lot #5192245) produced proteins. Full equivalence studies have been conducted for crops such as RR soybeans (Harrison *et al.*, 1993a), RR canola (Harrison *et al.*, 1993c), RR cotton (Burnette *et al.*, 1993) and RR corn (Lee *et al.*, 1995). These studies have confirmed that the plant-produced CP4 EPSPS protein was physically and functionally equivalent to the *E. coli*-produced CP4 EPSPS protein (lot #5192245) used to assess the safety of the CP4 EPSPS protein. N-terminal protein sequencing information has demonstrated that the chloroplast

transit peptide for each of these RR crops is properly cleaved yielding the "mature" CP4 EPSPS with the expected N-terminus. The specific enzymatic activities of CP4 EPSPS purified from these RR crop plants has also been shown to be equivalent to the specific enzymatic activity of the CP4 EPSPS protein isolated from *E. coli* (lot # 5192245). Additionally, the CP4 EPSPS protein produced in these RR crops was not glycosylated and showed comparable MW and immunological responses when detected using specific antibodies. These data, taken together, indicate that CP4 EPSPS protein expressed in a plant matrix has equivalent properties to those of the *E. coli* produced CP4 EPSPS (lot # 5192245) used for safety studies. This study represents a further extension of these previous equivalence results for the *E. coli*-produced CP4 EPSPS (lot # 5192245) to CP4 EPSPS produced in two RR corn lines NK600 and NK603.

B. PURPOSE

The purpose of this study was to assess the equivalence of 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4 (CP4 EPSPS) produced in *Escherichia coli* (*E. coli*) and in the two Roundup Ready® corn (*Zea mays* L.) lines NK600 and NK603. Because plant-expressed CP4 EPSPS has been extensively studied, this equivalence assessment was based on demonstration of comparable electrophoretic mobilities (i.e. comparable apparent MW) and immunological properties based on the results obtained by immunoblotting. The CP4 EPSPS protein as expressed by a commercially available soybean line (AG3701) was included for comparison, representing a plant biotechnology product for which the full equivalence of the *E. coli* (lot # 5192245) and plant-produced protein has been confirmed. Demonstration of equivalence justifies the application of the safety data generated using the *E. coli* produced protein for the CP4 EPSPS protein produced in Roundup Ready® corn events NK600 and NK603. Additionally, this study serves to characterize these Roundup Ready® corn events with respect to the CP4 EPSPS protein. This study will be part of the data submitted to regulatory agencies supporting the commercialization of Roundup Ready® corn plants containing the CP4 EPSPS protein.

III. MATERIALS

A. Test materials. The test materials were Roundup Ready® corn events NK600 and NK603 which endogenously express the CP4 EPSPS protein. Grain for these test lines was collected from field grown plants grown under Monsanto Production Plan 98-01-46-01. All grain was stored at approximately -20 °C or below. The identity of the test lines has been established by use of the polymerase chain reaction (PCR, Study # 99-01-46-38).

B. Control materials. There were two control materials for this study. Once control material was non-transgenic corn line (LH82 x B73) which does not contain the genetic material for CP4 EPSPS. Grain for the control line was collected from field grown plants as

specified by under Monsanto Production Plan 98-01-46-01. All grain for line (LH82 x B73) was stored at approximately -20 °C or below. The identity of the control line was established by use of the polymerase chain reaction (PCR, Study # 99-01-46-38).

The second control material was non-transgenic soybean line A5403 which does not contain the genetic material for CP4 EPSPS. The control grain sample was obtained from plants produced according to Study Protocol # 92-01-30-02 (Padgette *et al.*, 1993). Control soybean grain will be stored at approximately -20 °C or below. This second control material (A5403 soybeans) was added by amendment to the study protocol.

C. Reference materials. The first reference material was CP4 EPSPS protein produced by fermentation (100 L) of *E. coli* strain GB100 (Bishop, 1992), transformed with plasmid pMON21104 (Padgette *et al.*, 1993). The protein was purified to greater than 90% purity (Heeren *et al.*, 1993) by a combination of cell extraction, ammonium sulfate precipitation, hydrophobic and anion exchange chromatography. The *E. coli*-expressed CP4 EPSPS protein has been characterized (Harrison *et al.*, 1993b). This CP4 EPSPS standard (lot # 5192245) was stored in a buffer solution containing 50 mM Tris-HCl, pH 7.5, 150 mM KCl, 2 mM DTT and 50% (v/v) glycerol at approximately 3.96 mg/mL total protein. A comprehensive safety assessment of the CP4 EPSPS protein has been conducted (Harrison *et al.*, 1996). The stock solution of CP4 EPSPS protein was stored at -20 °C or below. Prior to the initiation of this study, the purity and immunoreactivity of CP4 EPSPS (lot #5192245) was reassessed and its suitability as a reference standard for immunoblotting confirmed. The purity of the CP4 EPSPS by image analysis of a Colloidal Brilliant Blue G-stained SDS-PAGE gel was estimated to be approximately 83%. Only one immunoreactive band was observed at the expected MW when 5 ng of total protein was analyzed.[1]

The second reference substance was CP4 EPSPS protein endogenously expressed by Roundup Ready® soybean line AG3701, obtained from Asgrow (Stonington, Illinois). Roundup Ready® soybeans have been approved by regulatory agencies in several countries including the U.S., Europe and Japan. The safety of CP4 EPSPS protein and Roundup Ready® soybeans has been confirmed (Harrison *et al.*, 1996; Padgette *et al.*, 1996). Chain of custody records were used to confirm the identity of line AG3701 soybeans. The soybeans were stored at room temperature or below.

IV. SUMMARY OF EXPERIMENTAL DESIGN

Corn and soybean grain extracts were prepared in an appropriate buffer solution and the extracts clarified by centrifugation. Corn extracts were subsequently concentrated approximately 2-fold. The total protein concentration of each sample extract was determined

[1]Data for the re-analysis of CP4 EPSPS purity and immunoreactivity was recorded in Monsanto red notebook pages 6458796-6458800 and 661430-6614307, respectively.

according to the method of Bradford (Bradford, 1976). Laemmli extracts were analyzed by Western blot methodology (following the appropriate SOPs) to assess the equivalence of CP4 EPSPS protein expressed in *E. coli*, RR soybeans and in Roundup Ready® corn lines NK600 and KN603 based on apparent MW (electrophoretic mobility) and immunological response when detected using specific antibodies.

V. TEST SYSTEM/ JUSTIFICATION OF TEST SYSTEM.

There was no test system for this study. The Western blot analytical procedure was employed to compare the physical and immunological properties of the CP4 EPSPS protein from RR corn, *E. coli* and RR soybean. The Western blot as employed in this study is highly sensitive, specific for CP4 EPSPS proteins, and allows for comparison of the apparent molecular weights of proteins possessing immunological cross-reactivity in complex mixtures.

VI. METHODS

A. Preparation of Grain Extracts. All extraction procedures were conducted on ice or at ~ 4 °C. Approximately 1 g of the corn and soybean samples was homogenized in ~6 mLs of a buffer solution containing: 100 mM Tris-Cl, pH 8.0, 1 mM EDTA, 5% (v/v) glycerol, 0.5 mM PMSF, 1% (w/v) PVPP, 1 mM benzamidine-HCl and 2 mM glutathione (reduced form). Homogenization was accomplished by use of a hand held tissue homogenizer (Omni 2000, Waterbury, CT) for 30 sec at speed setting of 4. After homogenization, extracts were centrifuged for 20 min at 12,000 rpm (20,800 x g) using an SA-600 rotor to clarify. The supernatant solutions for all corn protein extracts were concentrated approximately 2-fold using Ultrafree-4 (Millipore, Cat. No. UFV4BCC25) centrifugal concentration devices according to the manufacturer's instructions. An aliquot of each extract was mixed with an equal amount of 2X Laemmli buffer (Laemmli, 1970) and heated at ~100 °C for 5 min. Samples were stored at -80 °C until analyzed using the Western blot analytical procedure. Before subsequent analysis by SDS-PAGE and immunoblotting, the total protein concentration of an aliquot of each extract (not in 1X Laemmli buffer) was determined by the method of Bradford (Bradford, 1976) according to SOP GEN-PRO-015-00.

B. SDS-PAGE Analysis. Extracts prepared as above for electrophoresis were analyzed by sodium dodecyl sulfate polyacrylamide electrophoresis (SDS-PAGE) on 4-20% gradient gels according to the current version of SOP PB-EQP-005 using the mini gel system of NOVEX (San Diego, CA). Electrophoresis was conducted at constant voltage (approximately 150V) until the dye front reached the bottom of the gel. Extracts of NK600 and NK603 were analyzed on separate gels with an appropriate set of reference and control material extracts. Additionally, Amersham full-range color markers (RPN 800) were analyzed on each gel so that effective transfer to PVDF membrane could be confirmed. Finally, Bio-Rad biotinylated MW markers (Cat. No. 161-0319) were loaded on each gel so that blots could be calibrated.

C. Western Blot Analysis (immunoblotting). Immunoblotting was conducted according to the current version of SOP GEN-PRO-002. Proteins separated by SDS-PAGE were electrophoretically transferred to a PVDF membrane (Immobilon-P, 0.45 µM) and non-specific sites on blots were blocked using 5% non-fat dry milk in 1X TBST-[25 mM Tris-Cl, pH 7.5, 150 mM NaCl, 0.05% (v/v) Tween 20]. Blots were probed for the presence of CP4 EPSPS using a 1:1000 dilution of antisera raised in goats (DR2, bleed 5) against *E. coli*-produced CP4 EPSPS in 1X TBST with 1% (w/v) NFDM. Unbound polyclonal antibody was rinsed away with TBST washes. Polyclonal antibody bound to the membrane was probed with a 1:2000 dilution of biotinylated protein G (Pierce, P/N 29988) in 1X TBST with 1% (w/v) NFDM. Unbound biotinylated protein G was rinsed away with TBST washes. Biotinylated protein G bound to blots was detected using a 1:10,000 dilution of NeutrAvidin-HRP conjugate (Pierce, P/N 31001). Unbound NeutrAvidin-HRP was rinsed away with TBST washes. Immunoreactive bands were visualized on X-ray film using the Enhanced Chemiluminescence kits of Amersham (RPN 2106) according the instructions provided by the manufacturer. Films were developed using and automatic film processor (Konica SRX-101).

VII. RESULTS AND DISCUSSION

The *E. coli*-produced CP4 EPSPS used for safety studies, the CP4 EPSPS expressed by RR soybeans and corn lines NK600 and NK603 were found to be equivalent based on identical electrophoretic mobilities and detection using specific antibodies as established by the Western blot analytical method (see Figures 1 and 2). Results for NK600 are shown in Figure 1, and results for NK603 are shown in Figure 2. For both blots, the *E. coli* standard (lot # 5192245) was loaded at two concentrations (1 ng and 5 ng) in lanes 2 and 3, respectively. Additionally, *E. coli* CP4 EPSPS was spiked at 1 ng in both control corn (lane 5) and control soybean (lane 9) matrix to account for any possible bias associated with the relative mobility of CP4 EPSPS in plant extracts. The protein extracts prepared from the RR corn lines (NK600 and NK603) were loaded in lane 6 on separate gels, and the protein extract prepared from RR soybeans (line AG3701) was loaded in lane 7 of each gel. Stained bands at the expected apparent MW (~47 kD) were observed for the *E. coli*-produced CP4 EPSPS (whether alone or in plant matrix), the CP4 EPSPS in RR corn lines (NK600 and NK603) and in RR soybeans (line AG3701). No bands were detected in the control corn or soybean extracts, confirming the specificity of the antibodies. No other immuno-reactive bands were detected on these blot films. CP4 EPSPS has been expressed as a nuclear-encoded CP4 EPSPS with and an amino-terminal fusion to a chloroplast transit peptide (CTP2). The CTP is necessary for transport of the protein into the chloroplast, which is followed by cleavage of the CTP to yield the "mature" form of the protein. These results provide indirect evidence that the chloroplast transit peptide (CTP2) is properly processed upon chloroplast import yielding the "mature" CP4 EPSPS of the same size as that expressed in RR soybeans. As expected, these data clearly establish that the CP4 EPSPS expressed in RR corn lines NK600 and

NK603 is equivalent to the *E. coli* CP4 EPSPS protein used for safety assessment studies and is also equivalent to the CP4 EPSPS protein expressed in the commercial RR soybean variety AG3701.

VIII. CONCLUSIONS

CP4 EPSPS protein produced in Roundup Ready® corn lines NK600 and NK603 was demonstrated to be equivalent to both the *E. coli*-expressed CP4 EPSPS protein used for safety studies and CP4 EPSPS expressed by a commercial RR soybean variety. Equivalence was based on visually equivalent apparent molecular weights and immunological properties when detected using antibodies specific for CP4 EPSPS protein. This demonstration of equivalence justifies the application of the safety data generated using the *E. coli* produced protein for the CP4 EPSPS protein produced in Roundup Ready® corn events NK600 and NK603. This study will be part of the data submitted to regulatory agencies supporting the commercialization of Roundup Ready® corn plants containing the CP4 EPSPS protein. This study also served to characterize these Roundup Ready® corn lines with respect to the CP4 EPSPS protein.

IX. REFERENCES

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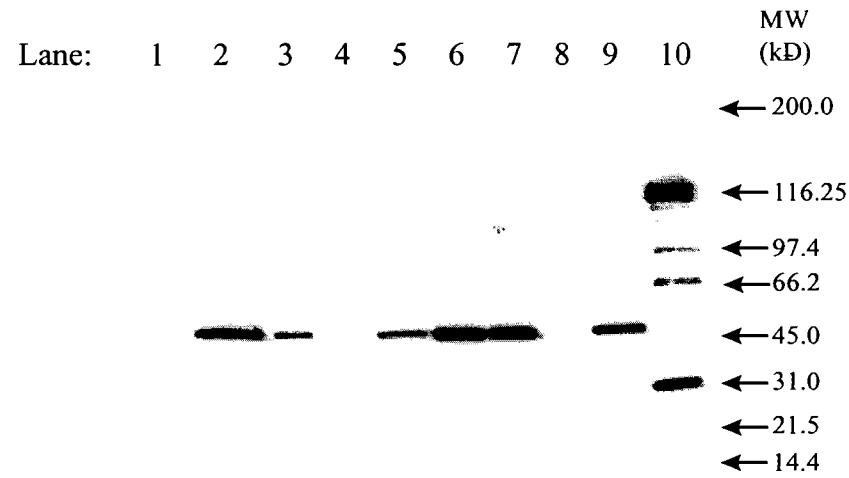
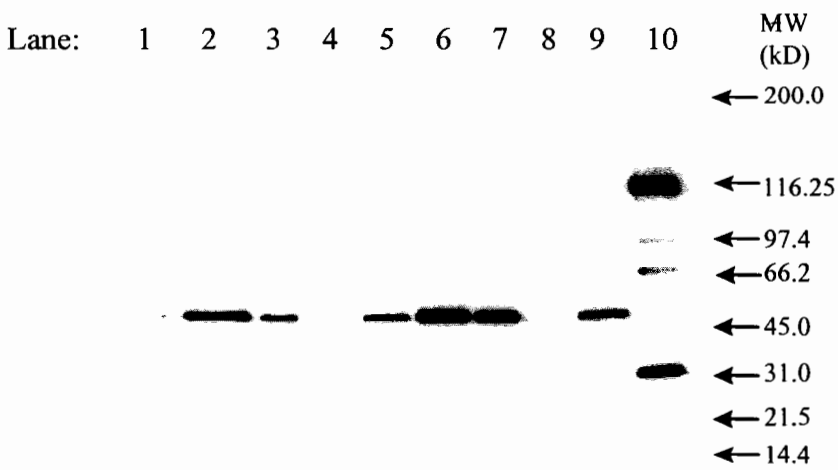
Figure 1. Western Blot Showing the Equivalence of CP4 EPSPS Protein Expressed by *E. coli*, Roundup Ready® Soybean and Roundup Ready® Corn Line NK600



Lane	Description	Amount
1	Full range color MW markers	7.5µL, 1.125 µg/band
2	*E. coli* CP4 EPSPS standard	10 µL, 5 ng
3	*E. coli* CP4 EPSPS standard	5 µL, 1 ng
4	Control corn extract (LH82 x B73)	8.4 µL, 10 µg total protein
5	Control corn extract (LH82 x B73) spiked with CP4 EPSPS	8.4 µL control extract and 2 µL (1 ng) standard
6	NK600 corn extract	9.17 µL, 10 µg total protein
7	AG3701 soybean extract	2 µL, 2.6 µg total protein
8	A5403 control soybean extract	2 µL, 2.2 µg total protein
9	Control soybean extract (A5403) spiked with CP4 EPSPS	2 µL control extract and 2 µL (1 ng) of standard
10	Biotinylated MW markers	7.5 µL

The soybean 1X Laemmli samples were diluted 10-fold before analysis because of the high expression level of CP4 EPSPS in Roundup Ready® soybean line AG3701.

Figure 2. Western Blot Showing the Equivalence of CP4 EPSPS Protein Expressed by *E. coli*, Roundup Ready® Soybean and Roundup Ready® Corn Line NK603



Lane	Description	Amount
1	Full range color MW markers	7.5µL, 1.125 µg/band
2	*E. coli* CP4 EPSPS standard	10 µL, 5 ng
3	*E. coli* CP4 EPSPS standard	5 µL, 1 ng
4	Control corn extract (LH82 x B73)	8.4 µL, 10 µg total protein
5	Control corn extract (LH82 x B73) spiked with CP4 EPSPS	8.4 µL control extract and 2 µL (1 ng) standard
6	NK603 corn extract	12.8 µL, 10 µg total protein
7	AG3701 soybean extract	2 µL, 2.6 µg total protein
8	A5403 control soybean extract	2 µL, 2.2 µg total protein
9	Control soybean extract (A5403) spiked with CP4 EPSPS	2 µL control extract and 2 µL (1 ng) of standard
10	Biotinylated MW markers	7.5 µL

The soybean 1X Laemmli samples were diluted 10-fold before analysis because of the high expression level of CP4 EPSPS in Roundup Ready® soybean line AG3701.

Attachment: SOP List

SOP Number	SOP Title
PB-EQP-005-01	SDS Polyacrylamide Gel Electrophoresis
GEN-PRO-015-00	Bio-Rad Protein Assay
GEN-PRO-002-03	Western Blot Analysis (Immunoblotting)