Importance of CP4 EPSPS Determination

CP4, a protein derived from the bacteria *Agrobacterium tumefaciens*, is expressed in certain genetically modified (GM) corn, cotton plants, and soybeans as Roundup Ready®. CP4 EPSPS (5-enolpyruvyl-shikimate synthase) allows for glyphosate-tolerant crops to grow and allows for farmers to develop weed-control methods to protect the crops.

Although initial testing showed low enviromental impact and toxicity, controversy has arisen over the long-term impact of GM crops on the environment and whether or not GM foods are safe for consumption. Several animal studies have shown serious health risks, such as cancer, infertility, endocrine disruption, reproductive and developmental toxicity, and changes in major organs including kidney and the gastrointestinal system, can be associated with the consumption GM products (1).

Efforts like the Non-GMO Project are raising awareness towards the growing number of problems associated with this technology and generating a larger public demand for manufacturers to label their products accordingly.

The Abraxis CP4 EPSPS ELISA Kit allows for the analysis of 46 samples in duplicates. The test can be performed in less than 90 minutes.

Performance Data

Standard Curve:

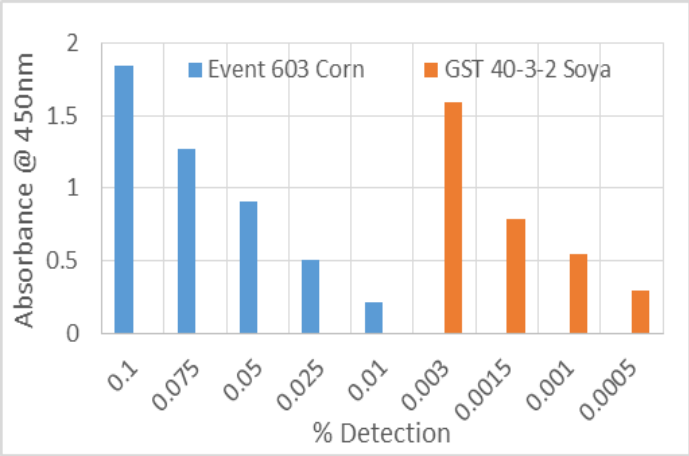
NOTE: Quantitative standard curve

Is for illustration purposes only and

To demonstrate assay sensitivity.

The kit is offered with positive and

Negative controls only.



For demonstration purposes only. Not for use in sample interpretation.

Pellet Pestles® is a registered trademark Kimble Chase Life Science and Research Products LLC.
Roundup Ready® is a registered trademark of Monsanto Technology, LLC.

Sample Prep. References:
USDA Grain Inspection Handbook, Book 1, Grain Sampling:
http://www.gipsa.usda.gov/fgis/handbook/gihbk1_inspec.aspx
Guidance document entitled Sampling for the Detection of Biotech Grains:
http://www.gipsa.usda.gov/fgis/biotech/sample2.htm
Practical Application of Sampling for the Detection of Biotech Grains:
http://www.gipsa.usda.gov/fgis/biotech/sample1.htm
Sample Planner Spreedsheet download:
www.gipsa.usda.gov/fgis/biotech/samplingplan1.xls

References:
(1) GMO Myths and Truths pgs 205-218:
http://responsibletechnology.org/irtnew/docs/GMO-Myths-and-Truths-edition2.pdf.

General Limited Warranty:
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CP4 EPSPS ELISA Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of
CP4 EPSPS in Corn, Cotton, Water, and Soybean Samples
Product No. 510101

1. General Description

Genentically modified seeds that expresses the CP4 EPSPS protein (or Roundup Ready®) can tolerate the herbicide glyphosate used by farmers to kill weeds without damaging the crops that have been genetically modified to be resistant to the herbicide. The Abraxis CP4 EPSPS ELISA is an immunoassay designed solely for use in the qualitative screening to detect the presence of CP4 EPSPS in corn, cotton, soybean seeds, water, soil, and leaf samples (please refer to the appropriate sample preparation or extraction). This test will detect at least 0.1% or one CP4 EPSPS seed in 1000 seeds. If necessary, positive samples can be confirmed by PCR or other conventional methods.

2. Safety Instructions

Discard samples according to local, state, and federal regulations.

3. Storage and Stability

The CP4 EPSPS ELISA should be stored in the refrigerator (4-8°C). The test strips, test vials, and samples to be analyzed should be at room temperature before use.

4. Test Principle

The test is a "sandwich" ELISA based on the recognition of CP4 EPSPS by specific antibodies. Controls, prepared samples and extracts are added to microtiter wells coated with anti-CP4 EPSPS monoclonal antibodies. The CP4 EPSPS in the controls and samples or extracts will be bound to the antibody coated wells. After a 30-minute incubation proceeded by a washing step, the "sandwich" is completed by the addition of an enzyme-labeled anti-CP4 EPSPS monoclonal antibody. The enzyme-labeled conjugated antibody is washed from the wells after a 30-minute incubation. After a final washing step, the substrate solution is added to produce a color signal. The intensity of the blue color is directly proportional to the concentration of the CP4 EPSPS present in the sample. The color reaction is stopped after 20 minutes and the color is evaluated using an ELISA plate reader. The sample is considered positive when the absorbance value is above the absorbance value of the negative control reagent.

5. Limitations of the CP4 EPSPS ELISA, Possible Test Interference

Numerous organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in samples, test interferences caused by matrix effects cannot be completely excluded.

Samples must be extracted and diluted as instructed in the sample preparation section (Section C) or appropriate technical bulletin before testing.

Mistakes in handling the test also can cause errors. Possible sources for such errors include: inadequate storage conditions of the test kit, incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme temperatures (lower than 10°C or higher than 30°C) during the test performance.

The Abraxis CP4 EPSPS ELISA kit provides screening results. As with any analytical technique (GC, HPLC, etc.), positive samples should be confirmed by an alternative method, such as PCR.

A. Reagents and Materials Provided

1. Microtiter plate coated with an anti-CP4 EPSPS monoclonal antibody, in a resealable aluminum pouch, 96 wells
2. CP4 EPSPS Positive Control Solution, 1 mL
3. Negative Control Solution, 1 mL
4. Anti-CP4 EPSPS monoclonal antibody-HRP conjugate, 4 vials (lyophilized)
5. Antibody conjugate diluent, 8 mL
6. Wash Solution (5X) Concentrate, 100 mL, must be diluted before use, see Test Preparation (Section D)
7. Extraction Solution/Sample Diluent (5X) Concentrate, 2 x 25 mL, must be diluted before use, see Test Preparation (Section D)
8. Substrate (Color) Solution (TMB), 12 mL
9. Stop Solution, 6 mL (handle with care)

B. Additional Materials (not delivered with the test kit)

1. Micro-pipettes with disposable plastic tips (20-1000 µL)
2. Multi-channel pipette (50-250 µL) or stepper pipette with disposable plastic tips (50-250 µL)
3. Microtiter plate reader (wave length 450 nm)
4. Timer
5. 5 mL microcentrifuge vials
6. Scoopula
7. Analytical 3 place balance (optional)
8. Pipette bulbs
9. Vortex mixer
10. 4 mL glass vials with Teflon-lined caps
11. Centrifuge capable of spinning at 3,000 rpm (1500 x g) (optional)
12. 15 mL or 50 mL centrifuge vials (optional)
13. Low protein binding syringe filter (0.8/0.2 µm Pall Acrodisc® PN 4905 or equivalent) with syringe (optional)
14. Small plastic bags or wax paper
15. Pliers, hammer, seed crusher, or disposable PELLET PESTLES® with microcentrifuge tubes (Kimble® PN 749520-0000 or equivalent)

C. Sample Preparation (To collect a composite sample according to the USDA/GIPSA guidelines, follow the links found in the "Sample Prep. References" in the final page of this insert.)

Corn,Cotton, Soyben Seed

1. Place a single seed in a small plastic bag and crush with a pliers or hammer (or place in seed crusher if available). Transfer the crushed sample to a 5 mL centrifuge vial. ***Note:*** *Take precautions to avoid sample cross-contamination. If a quantitative result is desired, the seed must be weighed.*
2. Add 4.0 mL of the 1X Extraction Solution (see Section D) to the centrifuge vial. Cap the vial and vortex for 30 seconds.
3. Let the sample settle for at least one minute.
4. The extract is ready to be analyzed (Section F. Assay Procedure, step 1).

Note: If a lower limit of dectection is needed, 1.0 mL of 1x Extraction Solution can be used.

Leaf Samples

1. Take 2-3 leaf punch samples by snapping the cap of the tube closed on the leaf. Determine the sample weight in mg. ***Note:*** *Take precautions to avoid sample cross-contamination. If a quantitative result is desired, the sample must be weighed.*
2. Grind the tissue by twisting and rotating the pestle in the tube until pulverized.
3. Add 500µL of 1X Extraction Solution/Sample Diluent to the tube.
4. Grind the tissue in the extraction solution by twisting and rotating the pestle in the tube (about 30 seconds).
5. Re-seal the microcentrifuge tube, vortex for 30 seconds, and let settle for at least 1 minute.
6. The extract is ready to be analyzed (Section F. Assay Procedure, step 1).

For **soil samples**, please refer to the appropriate technical bulletins.

Water Samples (water samples should be collected in glass jars with Teflon-lines caps and preserved immediately.)

1. Add 1 mL of 5X Extraction Solution/Sample Diluent for every 4 mL of water sample**, immediately** after sample collection. Mix well.
2. After samples are diluted, those samples containing gross particulate matter should be settled, centrifuged, or filtered using a syringe and low protein binding syringe filter (ex. 0.8/0.2 µm Pall Acrodisc® PN 4905 or equivalent).
3. Analyze as sample (Section F. Assay Procedure, step 1).

The CP4 EPSPS concentration in the water sample is determined by multiplying the ELISA result by a factor of 1.25. Highly contaminated samples, those outside of the calibration range of the assay, must be diluted further with 1X Extraction Solution/Sample Diluent and re-analyzed.

D. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the controls and the samples are necessary. In order to equalize the incubation periods on the entire microtiter plate, a multi-channel pipette or a stepping pipette is recommended for adding the enzyme conjugate, substrate, and stop solutions. Please only use the reagents and controls from one package lot in one test, as they have been adjusted in combination.

1. Dilute the Extraction Solution/Sample Diluent (5X) Concentrate at a ratio of 1:5 (i.e. 1 mL of solution added to 4 mL of deionized or distilled water and mix thoroughly) before extracting seed/leaf tissue samples or diluting samples/extracts. *Do not dilute if using to preserve water samples, see Sample Preparation (section C, Water Samples).*
2. Adjust the microtiter plate and the reagents to room temperature before use.
3. Remove the number of microtiter plate strips required from the aluminum pouch. The remaining strips are stored in the aluminum pouch and zip-locked closed. Store the remaining kit in the refrigerator (4-8°C).
4. The controls, substrate, and stop solutions are ready to use and do not require any further dilutions.
5. The HRP conjugate provided is lyophilized (2 vials). Once reconstituted, the conjugate solution will only remain viable for 4 weeks if stored at –20°C. If additional samples are to be analyzed greater than the listed shelf, a new vial of conjugate must be prepared. To reconstitute, **add 3 mL of Antibody Conjugate Diluent** to each vial of conjugate required and vortex. If more than 1/4 of plate needs to be run, then combine the reconstituted HRP conjugate vials, vortex slowly and add to plate as described in the Assay procedure (Section F).
6. Dilute the Wash Solution (5X) Concentrate at a ratio of 1:5. If using the entire bottle (100 mL), add to 400 mL of deionized or distilled water and mix thoroughly.
7. The stop solution must be handled with care as it contains diluted H_2SO_4.

E. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards that have been determined in a test performed previously.

Positive Control (Pos)

Negative Control (Neg)

Samp1, Samp2, etc.: Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	Pos	etc										
B	Pos	etc										
C	Neg											
D	Neg											
E	Samp1											
F	Samp1											
G	Samp2											
H	Samp2											

F. Assay Procedure

1. Add 100 µL of the **positive control, negative control, and samples** or **sample extracts** (Section C) into the wells of the test strips according to the working scheme given. Analysis in duplicate is recommended. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents.
2. Incubate the strips for 30 minutes at room temperature.
3. Remove the covering and decant the contents of the wells into a sink. Wash the strips **three times** using the **diluted washing buffer solution**. Please use at least a volume of 250 µL of washing buffer for each well in each washing step. Remaining buffer in the wells should be removed by patting the inverted plate dry on a stack of paper towels.
4. Add 100 µL of **Enzyme-conjugated anti-CP4 EPSPS Antibody Solution** to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents.
5. Incubate the strips for 30 minutes at room temperature.
6. Remove the covering and decant the contents of the wells into a sink. Wash the strips **three times** using the **diluted washing buffer solution**. Please use at least a volume of 250 µL of washing buffer for each well in each washing step. Remaining buffer in the wells should be removed by patting the inverted plate dry on a stack of paper towels.
7. Add 100 µL of **substrate (color) solution** to the wells. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for 20 minutes at room temperature. Protect the strips from direct sunlight.
8. Add 100 µL of **stop solution** to the wells in the same sequence as for the substrate solution.
9. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of stopping solution.

G. Evaluation

The interpretation of the qualitative results can be derived by simple comparison of the sample absorbances to the absorbances of the controls. Samples with absorbances that are above 0.200 are considered positive for CP4 EPSPS. Samples with absorbances below 0.200 are negative for CP4 EPSPS.

The mean absorbance of the negative control should not exceed the OD of 0.200. A valid test is when the positive control absorbance is above 0.200 and the negative control absorbance is below 0.200.

The coefficient of variance (%CV) between replicate positive control wells and negative control wells should not exceed 10%.

To calculate the %CV:

$$\%CV = \frac{\text{Standard Deviation OD}}{\text{Mean Pos. or Neg. OD}} \times 100$$