Importance of CP4 EPSPS Determination

CP4 EPSPS is the gene that is responsible for conferring resistance to the herbicide Glyphosate, most commonly known as the active ingredient in Roundup®, produced by Monsanto (now owned by Bayer). Glyphosate is a broad-spectrum herbicide that acts by disrupting the shikimic acid pathway found in plants, fungi, and bacteria. It disrupts this pathway by binding to the EPSPS enzyme, disabling it from functioning, which will then cause the plant to die from starvation. First introduced in 1974 by Monsanto under the trade name Roundup®, Glyphosate is the active ingredient in many different herbicide products sold under a variety of product names. Because it is a non-selective herbicide, the use of Glyphosate was initially limited to vegetation control in areas where it would not come into contact with food crops or other desirable vegetation and for grain desiccation during harvesting, in which it is applied to fields prior to harvesting to increase crop yields. Once Glyphosate began to be widely used, some microorganisms were found to be resistant to its effects and the genes which conferred this resistance were then identified. Using the *Agrobacterium* strain CP4, scientists at Monsanto genetically engineered those genes and inserted them into soybeans. These Glyphosate tolerant soybeans were branded as Roundup Ready® and were the first of a number of different Roundup Ready® seeds developed by Monsanto. With the ability to withstand the effects of Glyphosate, farmers were then able to apply Glyphosate for weed control in their fields during the growing season without harming their crops.

The determination of whether seeds contain the CP4 genes is important for several reasons, both to those seeking to use this trait in their farming practices and to those seeking to produce crops without the use of genetic modification and/or the use of herbicides and pesticides. For those attempting to avoid the use of genetically modified crops, this is becoming increasingly challenging, as contamination with the Roundup Ready genes is becoming more widespread.

Performance Data

Test sensitivity: The Abraxis CP4 EPSPS Strip Test for soybeans will detect the presence of the CP4 EPSPS protein present in Roundup Ready soybeans in samples containing one Roundup Ready soybean in 1000 conventional soybeans (0.1%). At this level, the test line will be visible. At levels less than 0.1%, the test line is not visible.

Samples: To detect 0.1% Roundup Ready® soybeans at 95% confidence, it is necessary to have 3 subsamples of 1000 beans each and all three subsamples should test negative (the weight of 1000 beans is 150 g).

Roundup® and Roundup Ready® are registered trademarks of the Monsanto Company.

General Limited Warranty: Abraxis, Inc. warrants the products manufactured by the Company, against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product's printed expiration date. **Abraxis, Inc. makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.**

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CP4 EPSPS Strip Test

Immunochromatographic Strip Test for the Detection of CP4 EPSPS Protein in Soybeans

Product No. 510200 (100 Test)

1. General Description

The Abraxis CP4 EPSPS Strip Test for soybeans is a rapid immunochromatographic test, designed solely for use in the qualitative screening of CP4 EPSPS in soybeans. The Abraxis CP4 EPSPS Strip Test provides only preliminary qualitative test results. Positive sample results should be confirmed by PCR, ELISA, HPLC or other conventional methods.

2. Safety Instructions

The CP4 EPSPS strip kit is intended for in vitro use only. The test strips contain the preservative thimerosol. Prevent direct contact of preservative with skin and eyes. Discard samples according to local, state, and federal regulations.

3. Storage and Stability

The CP4 EPSPS Strip Kit should be stored in the refrigerator at 2 – 8°C. The test strips must be stored in their original desiccated container with the cap firmly closed. The test strips, test vials, and samples to be analyzed should be at room temperature before use.

4. Test Principle

The test is based on the recognition of the CP4 EPSPS protein molecule by specific antibodies. An anti-CP4 EPSPS protein antibody is immobilized on the test line area of the nitrocellulose membrane on the test strip. A second anti-CP4 EPSPS protein antibody, which has been conjugated with colloidal gold, is incorporated into the sample area of the test strip. A control line, produced by a different antibody/antigen reaction is also present on the membrane strip. The control line is not influenced by the presence or absence of CP4 EPSPS protein in the sample extract and, therefore, should be present in all reactions.

When the test strip is placed in the sample extract, the CP4 EPSPS protein present in the sample extract binds to the antibody labelled with gold and the complex moves upward by capillary action. The complex is then bound by the antibody coated on the test line, resulting in the formation of a pink/purple test line. In the absence of CP4 EPSPS protein, no complex will be bound by the test line, and therefore no test line will be present. As the complex continues to migrate up the test strip, it is bound in the second antibody/antigen reaction also present on the membrane strip, resulting in a pink/purple color control line. As the control line is not influenced by the presence or absence of CP4 EPSPS protein in the sample extract, it will be present at the completion of the test procedure, indicating a valid test result.

5. Limitations of the CP4 EPSPS Strip Test, 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.

Mistakes in handling the test can also cause errors. Possible sources for such errors include:
Inadequate storage conditions of the test strip, too long or too short incubation times, extreme temperatures during the test performance (lower than 10°C or higher than 30°C), use of the test with samples types that have not been validated for use with the test kit.

The test is designed for use with soybean samples. The CP4 EPSPS Strip Test provides only a preliminary qualitative test result. Positive results should be confirmed by PCR, ELISA, HPLC or other conventional methods.

6. Warnings and Precautions

-Soybean samples must be prepared as described in section D (Sample Preparation/Extraction Procedure) before analyzing with the CP4 EPSPS strip test to produce accurate results.
-All materials, reagents, and samples should be allowed to reach room temperature before testing.
-Prior to use, ensure that the product has not expired by verifying that the date of use is prior to the expiration date on the label.
-The test strips are packaged in desiccated vials. The caps of the vials should be closed firmly after removing the required strips, as exposure to moisture will adversely affect the performance of the test strips.

-Avoid cross-contamination of sample extracts by using a new sample vial and disposable pipette for each sample and by thoroughly cleaning (washing and drying) the blender jar used for grinding soybean samples between each sample. Failure to adequately clean the blender jar may contaminate subsequent samples, producing inaccurate results.
-Use reasonable judgment when interpreting the test results.
-Results should be interpreted within 10 minutes after completion of the test.

A. Materials Provided

1. CP4 EPSPS test strips in a desiccated container (50 strips per container, 2 containers per kit)
2. Graduated disposable pipettes (100)
3. Microcentrifuge tubes (100)
4. User's guide

B. Additional Materials (not provided with the test)

1. Analytical balance
2. Blender or food processor
3. Deionized or distilled water
4. Graduated cylinder (1L)
5. Glass jars (1L)
6. Timer

C. Sample Collection

To detect 0.1% Roundup Ready® soybeans at 95% confidence, it is necessary to have 3 subsamples of 1000 beans each and all three subsamples should test negative (the weight of 1000 beans is 150 g).

A guideline on sampling strategy ("Practical Application of Sampling for the Detection of Biotech Grains") can be found at https://www.gipsa.usda.gov/fgis/biotech/sample1.htm. A sample planner spreadsheet can be downloaded from www.gipsa.usda.gov/fgis/biotech/samplingplan1.xls.

D. Sample Preparation/Extraction Procedure

Soybean samples must be prepared as described in the procedure below before testing:

1. Weigh and add the necessary quantity of soybeans to blender jar (see section C, Sample Collection, above for additional information on sampling).
2. Place cover on blender jar and grind sample at high speed for 45 seconds or until sample has a fine powder consistency.
3. Transfer ground sample to a clean, appropriately labelled glass jar.*
4. Determine the necessary volume of water for sample extraction (5 mL per 1 g of sample) using the following formula:

   _____ g of sample x 5 = _____ mL of water
5. Using the graduated cylinder, measure the appropriate volume of water (determined in step 4) and add to the ground sample in the glass jar.
6. Shake the jar vigorously to thoroughly mix the sample/water solution. Allow the solution to settle.
7. The upper liquid layer is now ready for testing (see section E, Procedure, below).

*Note: *Alternately, sample extraction may be performed in the glass blender jar, if desired. To perform extraction in the glass blender jar, omit step 3 (transferring the ground sample) and proceed to steps 4 – 7, using the blender jar as the extraction container. The final sample extract solution can then be transferred to a separate sample container, if desired.*

E. Procedure

Allow all reagents to reach room temperature before use. Sample extracts (prepared in section D, above) must be at room temperature before testing. Materials and sample extracts which are not at room temperature at the time of testing may produce inaccurate results.

1. Using a new disposable graduated pipette for each sample, transfer 0.5 mL of the upper liquid layer of the sample extract (prepared in section D, above) to a clean microcentrifuge tube.
2. Insert one test strip (arrows down) into the microcentrifuge tube.
3. Allow the test to develop for 10 minutes.
4. Remove the test strip. Lay the strip flat and read the results visually, as explained below in section F, Interpretation of Results.

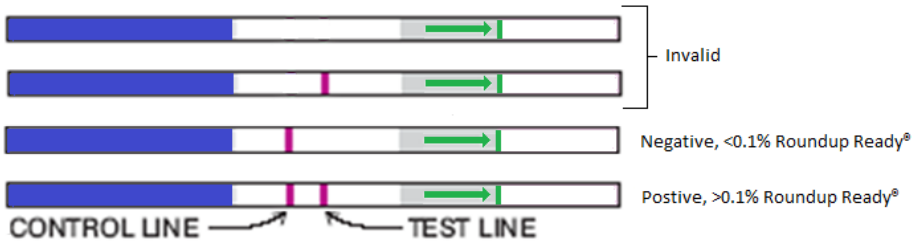
F. Interpretation of Results

Sample concentrations are determined by evaluating the intensity of the test line on the test strip. Results should be interpreted immediately upon the completion of the test (while the test strip is still wet). Determination made using strips which have begun to dry may be inaccurate, as line intensities may vary with drying time. Please note that although control line intensity may vary, a visible control line must be present for results to be considered valid. If no control line is visible within 10 minutes of the completion of the test, the test should be considered invalid and should be repeated with a new test strip to obtain valid results.

If a sample contains at least 0.1% Roundup Ready® soybeans (one Roundup Ready® soybean in 1000 conventional soybeans), the test line will be visible, indicating a positive result. Please note that the appearance of a faint test line after the strips have begun to dry may not indicate a positive result (results should always be interpreted immediately after the completion of the test to ensure accurate results). If a sample contains less than 0.1% Roundup Ready® soybeans, no test line will be visible, indiciating a result that is below the limit of detection of the test (negative result).

Control Line	***Test Line***	***Interpretation***
No control line present	No test line present	Invalid result
No control line present	Test line present	Invalid result
Control line present	No test line present	Negative, <0.1% Roundup Ready®
Control line present	Test line present	Positive, >0.1% Roundup Ready®

The appearance of test strips may also be compared to the illustration below to determine sample results.



Alternately, test strips can also be interpreted using the AbraScan test strip reader (PN 475025B), which provides objective determination of line intensities for consistent interpretation of results as well as a digital photographic record of all test strips.

G. Additional Analysis

Positive samples should be confirmed by PCR, ELISA, HPLC or other conventional methods. These services are available from commercial analytical laboratories (list of analytical laboratories available upon request).