

Roundup Ready (CP4 EPSPS) Test Kit

Instructions For Use

Format: Strip

Specimen: Extract of Soybean, Corn,
Cotton and other Crops

Catalog Number: 07-05-413



INTENDED USE

Artron CP4 EPSPS Test Kit for Roundup Ready Crop is intended to test the presence of CP4 EPSPS protein at a level typically expressed in genetically modified (GM) Roundup Ready Crop (Soybeans, Corn, Cotton, etc) to assist the determination of Roundup Ready Crops. The sensitivity of the test is 1 ng/ml, or 0.1% (i.e. one Roundup Ready seed in 1000 conventional kernels).



This assay provides only a preliminary result. Professional judgment should be sought to further evaluate the result of the test, particularly in evaluating a preliminary positive result.

SUMMARY AND PRINCIPLE OF THE ASSAY

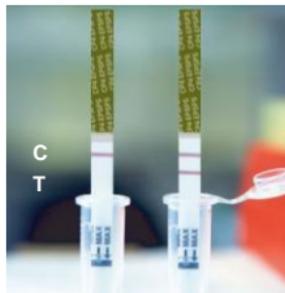
CP4 EPSPS is the protein introduced by genetic manipulation that is expressed in glyphosatetolerant soybeans, which are being developed to provide new weed-control options for farmers. Expression of this protein in plants imparts high levels of glyphosate tolerance. The safety of CP4 EPSPS was ascertained by evaluating both physical and functional characteristics. CP4 EPSPS degrades readily in simulated gastric and intestinal fluids, suggesting that this protein will be degraded in the mammalian digestive tract upon ingestion as a component of food or feed.

Artron CP4 EPSPS Test Kit for Roundup Ready Crop is an antigen-capture immunochromatographic assay, which detects the presence of CP4 EPSPS in extract of soybeans or other crop sample. Monoclonal antibodies specifically against CP4 EPSPS are 1) conjugated with colloidal gold and deposited on the conjugate pad, and 2) immobilized on the test line of the nitrocellulose membrane. Artron CP4 EPSPS Test Strip has an absorbent pad at each end. The protective tape with the arrow indicates the end of the strip to insert into the samples.

In order to detect the CP4 EPSPS protein expressed by Roundup Ready Crops, the sample must first be grounded to extract the protein. After the strip inserted into the sample,

the gold-antibody conjugate is rehydrated and the CP4 EPSPS, if any in samples, interacts with the gold conjugated antibodies. The antigen-antibody-gold complex will migrate toward the test window until the Test Zone (T) where it will be captured by immobilized antibodies, forming a visible pink line (Test band), indicating a positive result. If CP4 EPSPS is absent in the sample, no pink line will appear in the Test Zone (T), indicating a negative result.

To serve as an internal process control, a control line should always appear at Control Zone (C) after the test is completed. Absence of a pink control line in the Control Zone is an indication of an invalid result.



PACKAGE CONTENTS

- 100 Artron CP4 EPSPS Test Strips packed in two moisture-resistant canisters
- 1 Instructions for use

MATERIALS REQUIRED (BUT NOT PROVIDED)

- Clean, dry collection container (plastic or glass).
- Clock or timer.
- Sample grinder.
- Pestle (for leaf samples only)
- Tap water

WARNINGS AND PRECAUTIONS

- Do not reuse.
- Do not use if the pouch seal or its packaging is compromised.
- Do not use after the expiration date shown on the pouch.
- Do not mix and interchange different specimens.
- Wash hands thoroughly after finishing the tests.
- Do not eat, drink or smoke in the area where the specimens or kits are being handled. □ Keep out of children's reach.

SPECIMEN PREPARATION

1. Determine Sub-Sample Weight, and Grind Times

- Determine average weight of individual grain to be tested (weigh 100 beans divide by 100).
- Calculate the weight of the number of grains to be tested (Number of grains x Average Weight/Grain). Table 2 lists the guidelines for grinding time according to sample weight.

Table 2: Grinding time and sample weight.

Commodity	Sample Weight (g)	Grind Time (Sec)
Soybeans	9-15	20
	16-40	20
	41-60	30
	61-150	45

2. Determine the Volume of Water to be Used in Extraction

The test is suitable for testing both leaf tissue and seed of Soybean, corn, cotton and other crops. Leaves, or seeds must be grinded and diluted with water (Tap water could be used). For best result, different samples should be diluted with water in different ratio (see table below). Let the paste set for at least 45 seconds before testing with the test kits.

To extract seed:

A single seed sample can be grinded and then extracted with water in a tube. The sample preparation is a crucial procedure for the proper function of the test. The ratio of water in milliliters (ml) to the weight of the seed sample (g) ranges from 1:5 to 1:25 depends on the different crop.

To extract leaf tissue:

Take a 1-inch x 1-inch leaf punches from the sample leaf tissue; push the leaf punches into the tube by a pestle. Then squeeze 10 drops of water into tube and grind the tissue using the pestle.

Table 3: Sample Weight and Water Ratio

	Weight of Sample (g)	Volume of Water (ml)	Ratio weight (g):water (ml)

Soybean seed	20	100	1:5
Corn seed	20	100	1:5
Cotton seed	5	100	1:20
Single Corn Leaf	5	100	1:20
Multiple (composite) Leaves	10	100	1:10

3. Prepare the Sample

- Weigh sample into the appropriate size glass or plastic jar.
- The sample can be ground either dry or in water depend on the grinder available.
- Grind sample with a blender (on high speed) for specified grinding time or until all whole grains are broken into powder.
- Add the volume of tap water calculated according to Table 3 above.
- Cap and shake jar vigorously until the entire sample is wet (20-30 seconds, depending on the number of grains). Sample will begin to settle immediately and liquid can be drawn off at that time.
- Transfer of about 1 ml of the liquid of the sample to the reaction vial with the transfer pipette. (Draw up enough liquid portions from above the settled sample to fill the long narrow tip of the transfer pipette and up to the line at the top of the flared portion of the pipette bulb). Avoid pulling up particles.

Important: To prevent cross-contamination thoroughly clean blender parts and jars of dust and residue prior to preparation of a second sample. Use a new transfer pipette and reaction vial for each sample.

TEST PROCEDURES

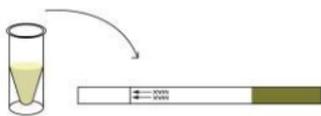
- 1 Allow refrigerated canisters to come to room temperature before opening. Remove the Artron CP4 EPSPS Test Strips to be used. Reseal the canister immediately.



- 2 Hold the strip at the colored end, and immerse the strip into the specimen in the reaction vial with the arrow end pointing towards the specimen. Do not immerse past the MAX line.



- 3 You could leave the strip in the reaction vial until the test is completed, or take the strip out after a minimum of 10 sec. Lay the strip (MAX side facing up) flat on a clean, dry, non-absorbent surface.



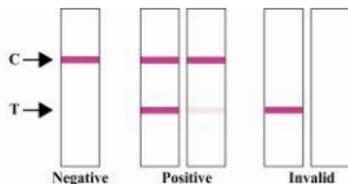
Read the result in 5 minutes Read results as shown under interpretation of Results

NOTE: Specimens with high concentrations of CP4 EPSPS may produce positive result in as little as 1 minute. Confirm negatives in 10 minutes.



**DO NOT INTERPRET RESULTS
AFTER 30 MINUTES**

RESULT INTERPRETATIONS



Negative

A pink band appears only at the control region, indicating absence of CP4 EPSPS or the concentration of the CP4EPSPS in the sample is below detection level (1 ng/ml).

Positive

Two pink bands appear at the control and test regions, indicating a positive result for CP4 EPSPS

Invalid

No visible band appears at the control region. Repeat with a new test kit. If the test still fails, please contact the

distributor with the lot number.

QUALITY CONTROL

Although the testing device contains an internal quality control (pink colored line in the control region), good laboratory practice recommends the daily use of an outside control to ensure proper testing device performance. Quality control samples should be tested according to the standard quality control requirements established by your laboratory.

STORAGE AND STABILITY

- Test device in the sealed pouch should be stored at 2-30°C. Do not freeze the test device.
- The test device should be kept away from direct sunlight, moisture and heat.

LIMITATIONS

- Humidity and temperature can adversely affect results.
- The instructions for the use of the test should be followed during testing procedures.
- There is always a possibility that false results will occur due to the presence of interfering substances in the specimen or factors beyond the control of the manufacturer, such as technical or procedural errors associated with the testing.
- Although the test demonstrates superior accuracy in detecting CP4 EPSPS, a low incidence of false results can occur. Therefore, other available tests are required in case of questionable results.

REFERENCES

1. <http://www.archive.gipsa.usda.gov/reference-library/handbooks/graininsp/grbook1/bk1.pdf>
USDA Grain Inspection Handbook, Book 1, Grain Sampling.

2. <http://www.archive.qipsa.usda.gov/biotech/sample2.htm>- Guidance document entitled Sampling for the Detection of Biotech Grains.
3. <http://www.archive.qipsa.usda.gov/biotech/sample1.htm>- Practical Application of Sampling for the Detection of Biotech Grains.
4. <http://www.archive.qipsa.usda.gov/biotech/samplingplan1.xls>- Sample Planner Excel Spreadsheet. The planner allows you to enter different assumptions in terms of sample size, number of samples, acceptable quality level and to determine the probability of accepting lots with given concentration levels.
5. Remund, K.M., Dixon, D.A., Wright D.L., Holden, L.R. "Statistical considerations in seed purity testing for transgenic traits", Seed Science Research, June 2001, Vol. 11 No.2, pp. 101-119.

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