- **INTENDED USE**

Artron GeneTrue Test Kit for Bt Cry1/CP4EPSPS Combo

Catalog Number: A07-15-413

Rapid Test User Instructions

The Artron GeneTrue Bt Cry1/CP4 EPSPS Combo Test is intended to determine the presence of the Bt Cry1Ab/Ac endotoxins and/or the CP4 EPSPS protein in genetically modified organisms.

Bt or *Bacillus thuringiensis* is a bacterium that produces toxins that are widely used as insecticides for their insecticidal properties. Due to these properties, Bt Cry1, a variation of the Bt toxin gene, has been introduced into plants by genetic manipulation, thus increasing the resistance of these genetically modified plants toward insecticides.

EPSPS or 5-enolpyruvylshikimate-3-phosphate synthase is an enzyme that catalyzes a reaction in plant and bacterial cells that is necessary for the synthesis of some amino acids. The herbicide, Roundup (glyphosate), can bind to the EPSPS enzyme made in plants and block its ability to work. This causes the plant to run out of amino acids which halts growth and development. The plant eventually dies from starvation. Roundup is a non-selective systemic herbicide which means it injures and often kills most plants that come into contact with it.

CP4 EPSPS is the protein introduced by genetic manipulation that is expressed in glyphosate-tolerant 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

Limit of detection: 1 ng/ml or 1 in 1000 kernels

- **STORAGE**

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

- **CONTENTS**

- Pouch (contains: test strip and desiccant).
- Test instructions.

- **MATERIALS REQUIRED (NOT PROVIDED)**

-Test tube
-Pestle (for leaf samples only)
-Seed crusher (for seed samples only)
-Tap water

- **SAMPLE PREPARATION**

Preparation of the sample is a crucial step for the test to run effectively. To prepare the sample, first grind the plant seeds/ leaves/ seedlings/ other materials that need testing. Mix the sample with water/buffer according to the predetermined ratio given below:

Plant component	Leaf (g) : Water (ml) **ratio for Plant component**	
	Leaf /Water (g/ml)	**Seed (g/ml)**
Corn	1 : 20	1 : 4
Cotton	1 : 10	1 : 4
Soybean	1 : 20	1 : 4

For plant components that are not listed below, please refer to the table and draw inferences by selecting the closest component category that would be most applicable for the sample. After completing the process of grinding the sample and adding water, ensure that the sample is mixed properly. This can be done by shaking or centrifuging the test tube. Once this has been accomplished, allow the mixture to stand for a while. It is recommended that you remove the liquid (supernatant) fraction of the ground plant mixture to another container for testing.

For single seed types:

Ground a single seed sample (0.5-2 g ground seeds) in a container and add water or buffer. Mix for 15–25 seconds and let the sample settle before testing.

For a mixture of different seed types:

Grind 200 g of mixed seeds. In a beaker, combine 20-50 g of the ground sample with water or buffer. Mix for 2-5 minutes and let the sample settle before testing.

For leaf tissue from the single plant type:

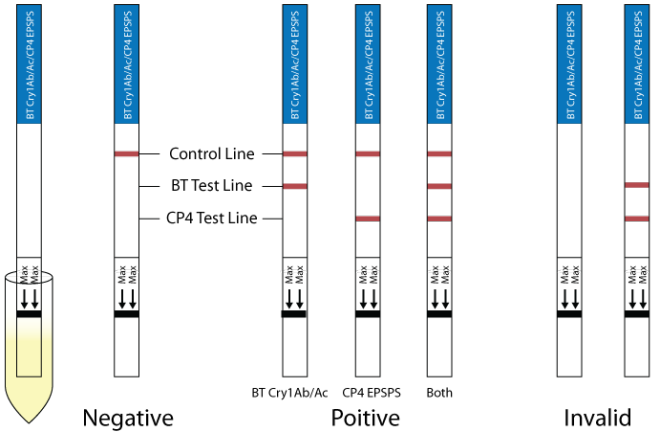
Take 1-inch x 1-inch leaf punches from the sample leaf tissue (0.15-0.3 g); push the leaf punches into the tube using a pestle. Add 3 mL of water or buffer into tube and grind the tissue using the pestle. This will result in a dilution of 1:20, if 0.15 g or 1:10, if 0.3 g. Let the paste settle for at least 45 seconds before testing or further dilution with water or buffer.

For leaf tissue from a mixture of different plant types:

Take 1-inch x 1-inch leaf punches from each of the different sample leaf tissues (total 0.2-0.4 g); push the leaf punches into the tube using a pestle. Add 4mL of water or buffer into tube and grind the tissue using the pestle. This will result in a dilution of 1:20, if 0.2 g or 1:10, if 0.4 g. Let the paste settle for at least 45 seconds before testing or further dilution with water or buffer.

- **HOW TO RUN THE STRIP TEST**
  - Remove the testing device from the foil pouch by tearing at the notch. Hold the strip at the colored end. Do not touch the arrow end or the test window (the middle part of the strip).
  - Holding the strip vertically, immerse the end of the strip with the arrows into the sample liquid. Do not immerse past the MAX line.
  - Take the strip out when the sample has migrated to the test window (about 10 seconds). Lay the strip (MAX side facing up) flat on a clean, dry, non-absorbent surface.
  - Wait 2-5 minutes for the result. If there is only one line appears, allow the strip to develop for an extra 10 minutes before completion of the test. Results visible after 30 minutes are considered invalid.

- **INTERPRETING THE RESULTS**
  - A **positive result** is the presence of purple bands: on the test (T) lines and one on the control (C) line.
  - A **negative result** is the presence of one purple band on the control (C) line.
  - An **invalid result:** no band is present on the control (C) line. This may suggest the procedure was done incorrectly or the test strip was faulty. Re-test with another strip. If the problem persists, please contact your local supplier.



For technical assistance or questions regarding the use of this test system, please call Artron Laboratories Inc.
Phone: 604-415-9757
Fax: 604-415-9795
Email: info@artronlab.com

- **FAQs**

1. **Organic seed samples are not climbing high enough to the test window. What should I do?**
   Organic seeds are capable of absorbing liquid. Therefore less liquid would be available in the supernatant for strip testing. To avoid this, centrifugation can be used to separate the liquid from the seeds. If a centrifuge is not available, you can increase the amount of samples and water/buffers (while maintaining the same ratio).

2. **What is the difference between water and buffer?**
   Buffer is a standard solution for laboratory testing. We recommend water because the average consumer might not have access to buffers. Buffers are more resistant to pH changes. This allows for more pH control for the user.

3. It can be difficult to decipher the results because there is a lot of redness that remains in the test windows. What should I do?

A red background along the membrane of a strip could be due to excess analyte in your sample. The site of the test/control bands are loaded with antibodies but the number is still finite. If your sample has more than there are binding sites, they may pool elsewhere/anywhere along the strip. You can try and clear the strip by dipping the tested strips in clear water. You can: (1) perform the regular test, (2) wait until the strip is dry and (3) dip the dried strip in clear water, following the same steps as with the sample. You can even repeat a second time if necessary.

LIMITED WARRANTY

Artron Laboratories Inc. warrants the products manufactured by Artron will be free of defects in materials and workmanship when used in accordance with the applicable instructions for a period of one and half year from the date of shipment of the products or if shorter, for a period not to extend beyond a product's printed expiration date.
The sole obligation of Artron shall be repair or replace the defective products in the manner and for the period above. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Artron be liable for any incidental, consequential, direct, indirect, or special damages