

Highlights:

- Results in 10 minutes or less
- Available as 100-strip individual kits, or bulk-packaged strips

Contents of Kit:

- 100 QuickStix Strips packed in two moisture-resistant canisters
- 100 Disposable Tissue Extractors, each consisting of a tube with punch cap and pestle (optional item with bulk packaging)
- EB2 Extraction Buffer

Items Not Provided:

- Repeating pipetter or other means of dispensing 0.5 mL

Contact EnviroLogix to order bulk-packaged kits. Bulk kits include EB2 Extraction Buffer Concentrate. To prepare 1 liter, mix 50 mL 20x Concentrate with 950 mL of distilled or deionized water. Store refrigerated when not in use; allow to come to room temperature before using.



Obtain Leaf Tissue



Grind Tissue

Catalog Number AS 011 LS

Intended Use

This EnviroLogix QuickStix Kit for Roundup Ready cotton leaf and seed is designed to extract and detect the presence of CP4 EPSPS protein at the levels typically expressed in genetically modified cotton plant tissue.

How the Test Works

Cotton plants and seeds that have been genetically modified with a glyphosate (Roundup®) - tolerant gene express CP4 EPSPS protein in their leaf and seed. To detect the CP4 EPSPS protein with the EnviroLogix QuickStix, the sample must first be extracted in tap water or buffer to solubilize the protein.

Each QuickStix Strip has an absorbent pad at each end. The protective tape with the arrow indicates the end of the strip to insert into the sample extract. The sample will travel up the membrane strip and be absorbed into the larger pad at the top of the strip. The portion of the strip between the protective tape and the absorbent pad at the top of the strip is used to view the reactions as described under "Interpreting the Results". Please avoid bending the strips.

Sample Preparation

Note: If Extraction Buffer has been refrigerated, allow it to warm up to room temperature before preparing samples.

To extract cotton leaf tissue:

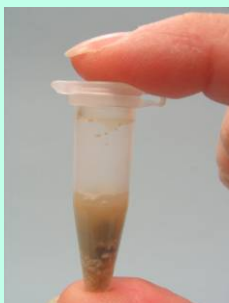
1. Sandwich a section of leaf tissue between the cap and body of the Disposable Tissue Extractor tube; snap two circular tissue punches by closing the cap. Push the leaf punches down into the tapered bottom of the tube with the pestle. Write the sample identification on the tube with a waterproof marker.
2. Insert the pestle into the tube and grind the tissue by rotating the pestle against the sides of the tube with twisting motions. Continue this process for 20 to 30 seconds or until the leaf tissue is well ground.
3. Add 0.5 mL Extraction Buffer.
4. Repeat the grinding step to mix tissue with water. Dispose of the pestle. Do not re-use pestles or tubes with more than one sample.

To extract cotton seed tissue:

1. Crush a single cotton seed (*Suggestion: use pliers with seed in microcentrifuge tube or resealable plastic bag*). Transfer to an extraction tube marked with sample identification. Note: Complete crushing of seed improves extraction efficiency and test performance.
2. Add 0.5 mL Extraction Buffer.
3. Close the tube cap securely. Shake the tube vigorously for 20 to 30 seconds, using an **up-and-down motion**, ensuring that the crushed seed and buffer are **well** mixed. Allow the solid material to settle to the bottom of the tube. The extract takes on a yellow to brown opaque color when the samples are prepared properly.



Crush single seed



Extract seed sample



Insert QuickStix



Any clearly discernable pink Test Line is considered positive

- Use caution to prevent sample-to-sample cross-contamination with plant tissue, fluids, crushing equipment (*pliers*) or disposables. Be sure to use a new tube for each sample tested.

How to Run the QuickStix Strip Test

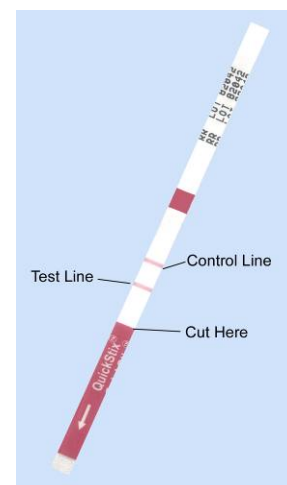
- Allow refrigerated canisters to come to room temperature before opening. Remove the QuickStix Strips to be used. Avoid bending the strips. Reseal the canister immediately.
- Place the strip into the extraction tube. The sample will travel up the strip. Use a rack to support multiple tubes if needed.
- Allow the strip to develop for 10 minutes before making final assay interpretations. Positive sample results may become obvious much more quickly.
- To retain the strip, cut off and discard the bottom section of the strip covered by the arrow tape.

Interpreting the Results

Development of the Control Line within five minutes indicates that the strip has functioned properly. Any strip that does not develop a Control Line should be discarded, and the sample re-tested using another strip.

If the extract is from a Roundup Ready seed, a second line (Test Line) will develop on the membrane strip between the Control Line and the protective tape. *The results should be interpreted as positive for CP4 EPSPS protein expression.*

If no Test Line is observed after 10 minutes have elapsed, the results should be interpreted as negative. A negative result means the sample contains less CP4 EPSPS protein than is typically expressed in the plant tissue of Roundup Ready modified cotton plants.



Kit Storage

This Kit can be stored at room temperature, or refrigerated for a longer shelf life. Please note the shelf life on the kit label for each storage temperature. The kit may be used in field applications; however, prolonged exposure to high temperatures may adversely affect the test results. Do not open the desiccated canister until ready to use the test strips.

Cross-reactivity

The following materials have been tested with this kit and have been found to cause no false positive results:

- Proteins: Cry1Ab, Cry1Ac, Cry1C, Cry1F, Cry2Ab, Cry3Bb, Cry9C, PAT/*pat*, PAT/*bar*, and cyanamide hydratase at concentrations at least 4 times higher than reported in commercial crops.
- Other GMO Event Cottons: Bollgard[®], Bollgard II[®], Bromoxynil resistant, and LibertyLink[®] cotton seeds.



- Other Crops: Seeds of conventional corn (yellow, white), soybean, and sugarbeet at 100% of sample.

Precautions and Limitations

- This kit is designed to screen for presence or absence only, and is not meant to be quantitative.
- As with all tests, it is recommended that results be confirmed by an alternate method when necessary.
- The assay has been optimized to be used with the protocol provided in the kit. Deviation from this protocol may invalidate the results of the test.
- The results generated through the proper use of this kit reflect the condition of the working sample directly tested. Extrapolation as to the condition of the originating lot, from which the working sample was derived, should be based on sound sampling procedures and statistical calculations which address random sampling effects, non-random seed lot sampling effects and assay system uncertainty. A negative result obtained when properly testing the working sample does not necessarily mean the originating lot is entirely negative for the analyte or protein in question.
- A strong positive result may safely be interpreted in as little as 2 minutes after sample addition. It is not safe, however, to interpret negative results prior to 10 minutes.
- Protect all components from hot or cold extremes of temperature when not in use. Do not leave in direct sunlight or in a vehicle.



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