

## Highlights:

- Recognizes Cry1Ac endotoxins
- 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 Envilab 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

Catalog Number AS 003 CTLS

## Intended Use

The EnviroLogix QuickStix Kit for Cry1Ac Cotton Leaf and Seed is designed to extract and detect the presence of the Cry1Ac Bt endotoxins at the levels typically expressed in genetically modified cotton plant tissue.

## How the Test Works

Cotton crops that have been genetically modified with a Bt gene express Bt endotoxins in their tissue. To detect these Cry1Ac proteins with this kit, tissue samples must be extracted and the endotoxins solubilized in the Extraction Buffer provided.

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 extraction tube. 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.”

## 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:

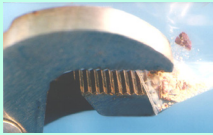
1. Sandwich a section of leaf tissue between the cap and body of the Disposable Tissue Extractor tube; snap two circular tissue punches by closing the cap. Push the leaf punches down into the tapered bottom of the tube with the pestle. Sample identification should be marked on the tube with a waterproof 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 Extraction Buffer. Dispose of the pestle (do not re-use pestles on more than one sample).

### To extract cotton seed:

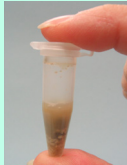
1. Crush a single cotton seed (*Suggestion: use pliers with seed in microcentrifuge tube or resealable plastic bag*). Transfer to an extraction tube marked with sample identification. Note: Complete crushing of seed improves extraction efficiency and test performance.
2. Add 0.5 mL Extraction Buffer.
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.
4. 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.



Grind Tissue



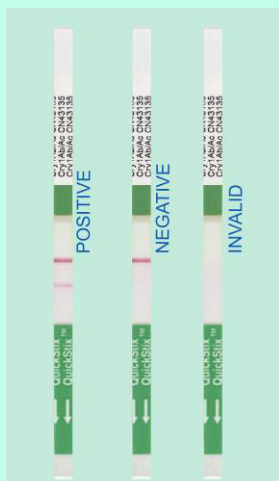
Crush single seed



Extract Seed Sample



Insert QuickStix



Any clearly discernible pink test line is considered positive

## How to Run the QuickStix Strip Test

1. 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.
2. Place the strip into the extraction tube. The sample will travel up the strip. Use a rack to support multiple tubes if needed.
3. Allow the strip to develop for 10 minutes before making final assay interpretations. Positive sample results may become obvious much more quickly.
4. To retain the strip, cut off and discard bottom section of the strip covered by the arrow tape.

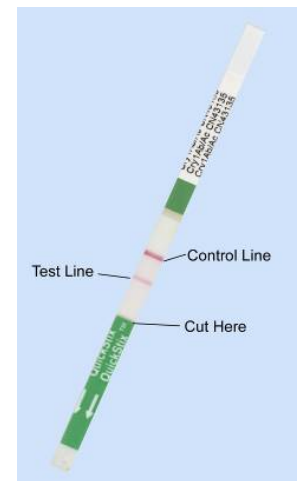
## Interpreting the Results

Development of the Control Line within 10 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 sample extract contained Cry1Ac endotoxin, a second line (Test Line) will develop on the membrane strip between the Control Line and the protective tape, within 10 minutes of sample addition. *The results should be interpreted as positive for Cry1Ac endotoxin expression. Any clearly discernible pink Test Line is considered positive.*

If no Test Line is observed after 10 minutes have elapsed, the results should be interpreted as negative, meaning that the sample contained less Cry1Ac endotoxin than is typically expressed in the tissues of Bt-modified plants.

**Warning:** A negative result with this test on cotton seed or leaf extracts does not necessarily rule out the presence of genetically modified material in the sample.



## Kit Storage

QuickStix can be stored at room temperature, or refrigerated for a longer shelf life. Note the shelf life on the kit box 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 using the protocols specified herein, and have been found to cause no false positive results at the levels indicated (concentrations are in leaf sample):

- Cry1C and Cry1F proteins at 100 ppm.
- Conventional, StarLink<sup>®</sup>, LibertyLink<sup>®</sup>, and Roundup Ready<sup>®</sup> Corn.
- Conventional and Roundup Ready soybean.
- Conventional and Roundup Ready sugar beet.
- Conventional and Roundup Ready canola.



## Precautions and Limitations

- This kit is designed for screening for presence or absence only and is not meant to be quantitative.
- As with all tests, it is recommended that results be confirmed with an alternate method if necessary.
- The assay has been optimized using the protocol and buffer 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 negative result with this kit does not mean that the sampled tissue has not been otherwise genetically modified.
- A strong positive result may safely be interpreted in as little as 2 minutes after sample addition. It is not safe, however, to conclude that a sample is negative before a full 10 minutes has elapsed, as a weak positive sample may require the full 10 minutes for a distinct Test Line to appear.
- Protect all components from hot or cold extremes of temperature when not in use. Do not leave in direct sunlight or in vehicle.

