ENVIRQLOGIX

QuickStix[™] Kit for Cry1Ab Corn Leaf & Seed

Highlights:

- Recognizes Cry1Ab endotoxins
- Results in 5 minutes or less
- Available as 100-strip individual kits, or bulkpackaged strips

Contents of Kit:

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

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.

Leaf testing



Obtain leaf tissue, grind

Catalog Number AS 003 CRLS

Intended Use

The EnviroLogix QuickStix Kit for Cry1Ab Corn Leaf and Seed is designed to extract and detect the presence of the Cry1Ab Bt endotoxins at the levels typically expressed in genetically modified corn plant tissue.

How the Test Works

Corn crops that have been genetically modified with a Bt gene express Bt endotoxins in their tissue. To detect these Cry1Ab 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. Fill the dropper bottle provided with Extraction Buffer.

To extract corn 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. Uncap the bottle of Extraction Buffer and invert it directly over the Tissue Extractor tube. Carefully squeeze 10 drops (0.5 mL) of Buffer into the tube.
- 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 corn seed:

- 1. Crush a single corn kernel (*Suggestion: Use pliers with seed in resealable bag*). Transfer to an extraction tube marked with sample identification.
- 2. Uncap the bottle of Extraction Buffer and invert it directly over the Tissue Extractor tube. Carefully squeeze **20 drops (1 mL)** of Buffer into the tube. Alternatively, remove the dropper tip from the bottle and dispense 1 mL of Buffer into the tube, using a pipette.
- 3. Close the tube cap securely and shake the tube vigorously for 20 to 30 seconds. Allow the solid material to settle to the bottom of the tube.

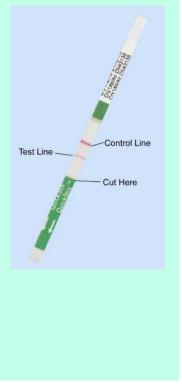
Seed testing



Crush single seed, extract



Insert QuickStix



4. Repeat the protocol for each sample to be tested, using a new tube and pestle for each. Use caution to prevent sample-to-sample cross-contamination with plant tissue, fluids, or disposables.

To extract bulk ground corn grain: For best results, please purchase the QuickStix Kit for Cry1Ab/Cry1Ac Bulk Grain, Cat# AS 003 BG.

How to Run the QuickStix Strip Test

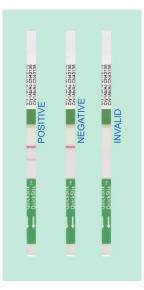
- 1. Allow refrigerated canisters to come to room temperature before opening. Remove the QuickStix Strips to be used. Avoid bending the strips. Reseal the canister immediately.
- 2. Place the strip into the extraction tube. The sample will travel up the strip. Use a rack to support multiple tubes if needed.
- 3. Allow the strip to develop for 5 minutes before making final assay interpretations. Positive sample results may become obvious much more quickly.
- 4. To retain the strip, cut off bottom section of the strip covered by the arrow tape.

Interpreting the Results

Development of the Control Line within 5 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 Cry1Ab endotoxin, a second line (Test Line) will develop on the membrane strip between the Control Line and the protective tape, within 5 minutes of sample addition. *The results should be interpreted as positive for Cry1Ab endotoxin expression.* Any clearly discernible pink Test Line is considered positive.

If no Test Line is observed after 5 minutes have elapsed, the results should be interpreted as negative, meaning that the sample contained less Cry1Ab endotoxin than is typically expressed in the tissues of Bt-modified plants.



Warning: A negative result with this test on corn <u>seed</u> or <u>leaf extracts</u> does not necessarily rule out the presence of genetically modified material in the sample.

Bt Events in Corn

Bt 11 Event Bt endotoxin is expressed in seed (grain) and plant tissue. **Bt 176 Event** Bt endotoxin is expressed only in the corn leaf.

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.





Precautions and Notes

- 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.
- Warning: 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 5 minutes has elapsed, as a weak positive sample may require the full 5 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.



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