QuickStix™ Kit for Cry1F Leaf & Seed

Catalog Number AS 016 LS

Highlights:

- *Results in 5 minutes or less*
- *Available as 100-strip individual kits, or bulk-packaged strips*
- *Also available in comb format for high-volume seed testing*

Contents of Kit:

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

Contact EnviroLogix to order bulk-packaged kits. Bulk kits include EB2 Extraction Buffer Concentrate. To prepare 1 liter, mix 50 mL of 20X Concentrate with 950 mL of distilled or deionized water. Store refrigerated when not in use; allow to come to room temperature before using. Expiration date for prepared buffer is equal to that stated on the 20x container.

Leaf tissue testing:

Obtain leaf tissue, grind

Intended Use

The EnviroLogix QuickStix Kit for Cry1F is designed to extract and detect the presence of the Bt endotoxin Cry1F found in Herculex™ I and Herculex™ XTRA (corn) and WIDESTRIKE® (cotton) Insect Protection traits. The QuickStix Kit will recognize the Cry1F protein in corn and cotton seed and leaf tissue extracts, with no cross-reactivity to any other commercially available GM proteins, including Cry34Ab1, Cry35Ab1 and PAT/*pat*. For Cry1F detection in bulk corn grain, please use QuickStix Cat. No. AS 016 BG.

How the Test Works

Corn and cotton crops that have been genetically modified with a c*ry1F* gene express Cry1F protein in their tissue. To detect the protein, 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 arrows indicates the end of the strip to insert into the sample extract. The sample travels up the membrane strip and is 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.*

Use *extreme* caution to prevent sample-to-sample cross-contamination with plant tissues, fluids, or disposables. Repeat the protocol for each sample to be tested using a new tube and toothpick. Avoid touching the pad at the bottom of the strip after handling leaf tissue.

A. To extract corn leaf tissue:

A1. Sandwich a section of **folded** leaf tissue between the cap and body of the Disposable Tissue Extractor tube. Snap **two** circular punches by closing the cap.

A2. Insert a 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.

A3. Holding the dropper bottle vertically, add **10 drops** or pipette **0.5 mL** of Extraction Buffer into the tube containing the leaf punch. Do not touch the dropper or pipette tip to the tube or leaf sample.

A4. Repeat the grinding step to mix tissue with Extraction Buffer. Tap the solid materials to the bottom of the tube. Dispose of the pestle (do not re-use pestles on more than one sample).

B. To extract cotton leaf tissue:

B1. Sandwich a section of leaf tissue between the cap and body of the Disposable Tissue Extractor tube. Snap **one** circular punch by closing the cap.

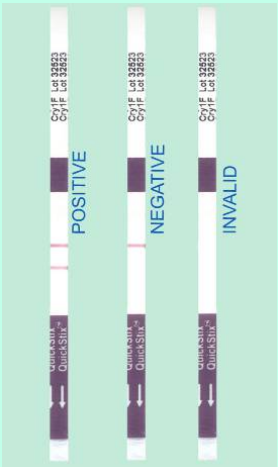
Add buffer, grind again

Seed tissue testing:

Crush seed

Extract seed sample

Insert QuickStix Strip



Any pink Test Line is considered positive

B2. Insert a 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.

B3. Holding the dropper bottle vertically, add **8 drops** or pipette **0.35 mL** of Extraction Buffer into the tube containing the leaf punch. Do not touch the dropper or pipette tip to the tube or leaf sample.

B4. Repeat the grinding step to mix tissue with Extraction Buffer. Tap the solid materials to the bottom of the tube. Dispose of the pestle (do not re-use pestles on more than one sample).

C. To extract corn or cotton seed tissue:

C1. Crush a single seed (Suggestion: use pliers with seed in resealable plastic bag). Transfer to a tissue extraction tube marked with sample identification.

C2. Holding the dropper bottle vertically, carefully dispense **20 drops** or pipette 1.0 mL of Extraction Buffer into the tube containing crushed corn kernel OR **10 drops** (0.5 mL) into the tube containing crushed cotton seed. Do not touch the dropper or pipette tip to the tube or seed sample.

C3. Close the tube cap securely. Shake the tube vigorously for **20 to 30 seconds.** After shaking step, tap the solid materials to the bottom of the tube.

C4. 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.

How to Run the QuickStix Strip Test

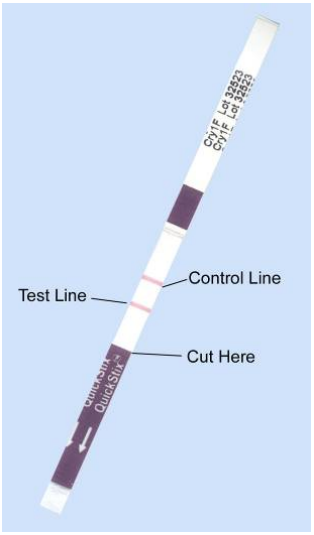
1. Allow refrigerated canisters to come to room temperature before opening. Remove the QuickStix Strips to be used. Avoid bending the strips. Reseal the canister immediately.
2. Place the strip into the extraction tube. The sample will travel up the strip. Use a rack to support multiple tubes if needed.
3. Allow the strip to develop in the extract for 5 minutes (if testing corn leaf/seed) or 10 minutes (if testing cotton leaf or seed) before making final assay interpretations. Positive sample results may become obvious much more quickly. Allow the test to run for the full testing time before concluding a sample is negative.
4. To retain the strip, cut off and discard the bottom section of the strip covered by the arrow tape.

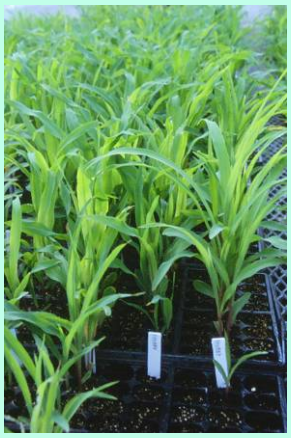
Interpreting the Results

Development of the Control Line within the testing time 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 contains Cry1F protein, a second line (Test Line) will develop on the membrane strip between the Control Line and the protective tape within 5 (corn) or 10 (cotton) minutes of sample addition. *The results should be interpreted as positive for Cry1F protein expression.*

If the extract is from a negative sample, the strip will only develop the Control Line.





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 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 (see "Precautions and Notes"). 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 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.
- Protect all components from hot or cold extremes of temperature when not in use. Prolonged exposure to high temperatures may adversely affect the test results. Do not leave in direct sunlight or in vehicle.
- 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 seed lot from which the leaf or seed 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.
- Age and condition of leaf tissue sampled can impact the intensity of the result.
- A strong positive result may be safely interpreted in as little as 2 minutes after sample addition. It is not safe, however, to conclude that a sample is negative before the full testing time has elapsed.
- Use extreme caution to prevent sample-to-sample cross-contamination with tissues, fluids, or disposables. Avoid touching the pad at the bottom of the strip after handling leaf tissue. Discard disposables after one use.

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License

EnviroLogix has developed this kit using proprietary reagents as well as reagents licensed from Pioneer (DuPont Agriculture & Nutrition) Company and Dow AgroSciences.

This test kit has been validated and approved by Dow AgroSciences for detection of their Herculex and WIDESTRIKE traits.

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