ENVIROLOGIX™

QuickStix™ Combo Kit for Cry1A & Cry2A Leaf & Seed

Highlights:

- *Recognizes Cry1Ac, Cry1Ab and Cry2A endotoxins*
- *Results in 10 minutes or less*
- *Available as 100-strip individual kits, or bulk-packaged strips*

Contents of Kit:

- *100 QuickStix Combo 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

Catalog Number AS 012 LS

Intended Use

The EnviroLogix QuickStix Combo Kit for Cry1A and Cry2A is designed to extract and detect the presence of these endotoxins at the levels typically expressed in genetically modified corn or cotton plant tissue and seed. The combo strips will recognize both Cry1A and Cry2A endotoxins in separate regions of the same strip.

How the Test Works

Crops that have been genetically modified with stacked Bt genes express Bt endotoxins in their leaf and seed. To detect these Cry1A and Cry2A proteins with the EnviroLogix QuickStix Combo Strip, the sample must be extracted and the proteins solubilized in the Extraction Buffer provided.

Each Combo Strip has an absorbent pad at each end. The protective tape with the arrow indicates which end of the strip to insert into the extraction tube. 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.*

To extract 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 Extraction Buffer. Dispose of the pestle (do not re-use pestles on more than one sample).

To extract seed:

1. Crush a single 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 to **cotton** seed – OR – add 1.0 mL Extraction Buffer to **corn** kernel.


Grind Tissue

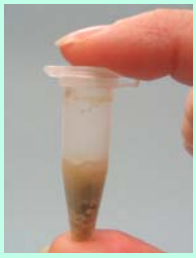
3. Close the tube cap securely. Shake the tube vigorously for 20 to 30 seconds, using an **up-and-down motion**, ensuring that the crushed seed and buffer are **well** mixed. Allow the solid material to settle to the bottom of the tube. The cotton seed extract takes on a yellow to brown opaque color 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.

How to Run the QuickStix Strip Test


Crush Seeds

1. Allow refrigerated canisters to come to room temperature before opening. Remove the Combo 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 the bottom section of the strip covered by the arrow tape.

Interpreting the Results

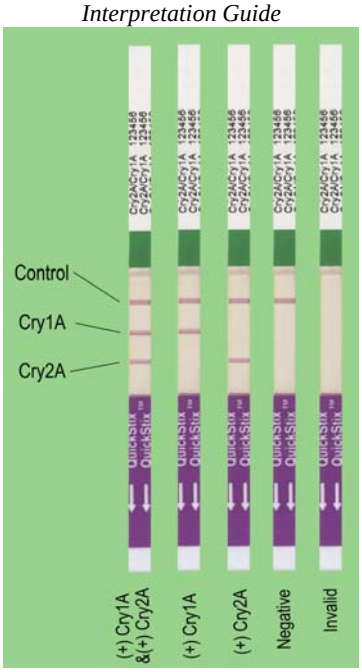

Extract Seed Sample

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.

One Line – If the extract is from a negative sample, the strip will only show the Control Line. 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.

Three Lines – If the extract is from a sample containing both Cry2A and Cry1A proteins, a total of three lines will appear. A Test Line for extracts containing Cry1A protein will appear about 5 mm below the control Line. A Test Line for extracts containing Cry2A protein will appear about 10 mm below the Control Line and approximately 5 mm below the Cry1A Test Line.

Interpretation Guide



Any clearly discernable pink Test Line is considered positive


Insert QuickStix

Two Lines – If the extract contains either Cry2A or Cry1A proteins, the strip will develop two lines. To identify the positive Test Line, compare the strip to the Interpretation Guide. Extracts containing Cry1A protein will exhibit a Test Line about 5 mm below the Control Line; extracts containing Cry2A protein will exhibit a Test Line about 10 mm below the Control Line.

Kit Storage

This QuickStix Kit 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 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.

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