QuickStix™ Kit for Cry2A Corn Leaf & Seed

Catalog Number AS 005 LS

Highlights:

- *Results in 5 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*
- *EB2 Extraction Buffer*
- *Dropper bottle*
- *100 Disposable Tissue Extractors, each consisting of a tube with punch cap and pestle (optional item with bulk packaging)*

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

Intended Use

The EnviroLogix QuickStix Kit for Cry2A is designed to extract and detect the presence of the Cry2A endotoxins at the levels typically expressed in genetically modified plant tissue.

How the Test Works

Crops that have been genetically modified with a Bt gene express Bt endotoxins in their leaf and seed. To detect these Cry2A proteins with this kit, tissue samples must be extracted and the endotoxin 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 leaf tissue:

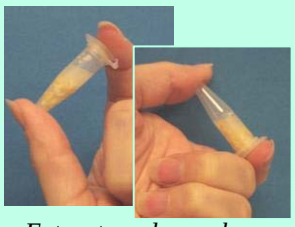
1. Sandwich a section of leaf tissue between the cap and body of the Disposable Tissue Extractor tube. Snap a circular tissue punch by closing the cap. Push the leaf punch down into the tapered bottom of the tube with the pestle. Write the sample identification on the tube with a waterproof marker.
2. 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. 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 containing leaf.
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. 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.
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 opaque color when the samples are prepared properly.

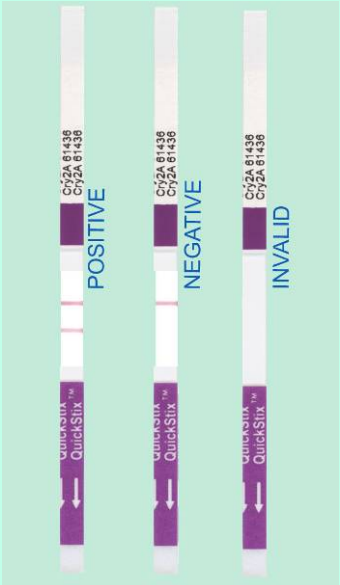
Seed testing

Crush single seed



Extract seed sample—vigorous shaking is important

Insert QuickStix



Any clearly discernable pink Test Line is considered positive

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

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.

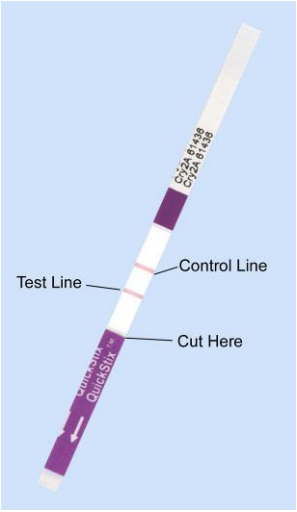
2. Place the strip into the extraction tube. The sample will travel up the strip. Use a rack to support multiple tubes if needed.

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 and discard the 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 contains Cry2A 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 Cry2A endotoxin expression.

If no Test Line is observed after 5 minutes have elapsed, the results should be interpreted as negative. A negative result means the sample contains less Cry2A endotoxin than is typically expressed in the tissues of Bt-modified plants.

Kit Storage

This QuickStix Kit should 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 taken into the field for use; however, prolonged exposure to high temperatures may adversely affect the test results. Important: do not open the desiccated canister until you are ready to use the test strips.

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 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 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 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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