

QuickStix[™] Kit for eCry3.1Ab Corn Leaf & Seed

Highlights:

- Results in 10 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 Buffer to warm to room temperature before use.





Obtain leaf tissue, grind



Add Buffer, grind again

Catalog Number AS 074 LS

Intended Use

The EnviroLogix QuickStix Kit for eCry3.1Ab Corn is designed to detect the presence of the eCry3.1Ab protein at levels typically expressed in genetically modified corn grain, leaf and seed tissues (Event 5307, Agrisure DuracadeTM). Corn derived from transformation Event 5307 contain the gene *ecry3.1Ab* which encodes eCry3.1Ab protein expression in the plant tissues. This protein is an engineered chimera of modified Cry3A (mCry3A) and Cry1Ab proteins. The kit will detect eCry3.1Ab protein in extracts prepared from the Event 5307 modified corn leaf and seed tissues.

How the Test Works

In order to detect the eCry3.1Ab protein with this QuickStix Kit, the sample must first be ground and extracted in 1X EB2 Extraction Buffer to solubilize the protein. 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 containing corn 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 corn seed:

- 1. Crush a single corn kernel (*Suggestion: Use pliers with seed in resealable bag*). Transfer to a Disposable Tissue Extractor marked with sample identification.
- 2. Uncap the bottle of Extraction Buffer and invert it directly over the Tissue Extractor tube. Carefully squeeze **20 drops** (**1.0 mL**) of Buffer into the tube.





Any pink Test Line is considered positive

Alternatively, remove the dropper tip from the bottle and dispense 1.0 mL (1000 microliters) into each 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.
- 4. Repeat the protocol for each sample to be tested, using a new tube 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 Tissue Extractor. 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.

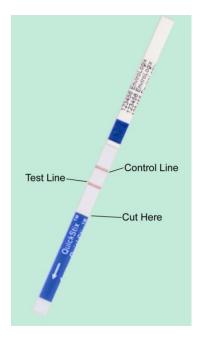
Interpreting the Results

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 leaf or seed extract is from a sample containing Event 5307, a second line (Test Line) will develop on the membrane strip between the Control Line and the protective tape. The results should be interpreted as positive for the presence of eCry3.1Ab protein.

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

Warning: A negative result with this test on corn seed or <u>leaf extracts</u> 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.



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 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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This test kit has been approved by Syngenta for detection of the eCry3.1Ab protein in Agrisure DuracadeTM traited corn.

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