

QuickStix™ Combo Kit for Multi-Trait Testing in Corn Leaf & Seed

Cry2A, 3B, 1F, 34

Highlights:

- Recognizes Cry2Ab, Cry3Bb, Cry1F, and Cry34 traits in a single corn seed
- 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
- 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 combs. Bulk kits include EB2 Extraction Buffer Concentrate. To prepare 1X EB2 Extraction Buffer, mix 50 mL 20X Concentrate with 950 mL of distilled or deionized water per liter required. Store refrigerated when not in use. *Buffer should be used at RT.

Leaf testing



Obtain leaf tissue, grind

Catalog Number AS 087 LS

Intended Use

The EnviroLogix QuickStix Combo Kit for Multi-Trait Testing is designed to extract and detect the presence of multiple traits expressed in genetically modified corn leaf or seed tissue. The combo strips will recognize Cry2Ab, Cry3Bb, Cry1F and Cry34 proteins in separate regions of the same strip, as expressed in SmartStax® Corn.

How the Test Works

Corn plants and seeds that have been genetically modified with multiple events will express transgenic proteins in their leaf and seed tissue. To detect these proteins with the Combo Strips, the sample must be extracted and the proteins solubilized in the Extraction Buffer. 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 sample. 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 a circular tissue punch by closing the cap. Repeat for a total of 2 leaf tissue samples (hint: fold a leaf and punch through for 2 samples). 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. 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).

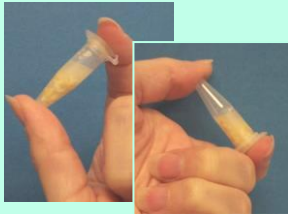
To extract seed:

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 (1.0 mL) of Buffer into the tube.

Seed testing



Crush single seed



Extract seed sample—
vigorous shaking is important



Insert QuickStix

Any clearly discernable pink
Test Line is considered positive

- 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.
- 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 Comb Test

- 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.
- Place the strip into the extraction tube. The sample will travel up the strip. Use a rack to support multiple tubes if needed.
- Allow the strip to develop for 10 minutes before making final assay interpretations. Positive sample results may become obvious much more quickly.
- 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.

One Line – If the extract is from a negative sample, containing none of the target proteins, the strip will only show a Control Line, about 4 mm below the top pad.

Two to Five Lines – If the extract is from a sample containing Cry2A, Cry3Bb, Cry1F, and/or Cry34 proteins, the appropriate test line(s) will appear. Please refer to the interpretation guide below.

Lot Number	Cry2Ab and Cry1A.105 (MON 89034)	Cry3Bb and CP4 EPSPS (MON 88017)	Cry1F (TC1507)	Cry34/35 (DAS 59122-7)	Indicates SmartStax	Conventional (non-target) Corn Seed
150611 34/1F/3Bb/2A	✓	✓	✓	✓	✓	✓
150611 34/1F/3Bb/2A	✓	✓	✓	✓	✓	✓
Control Line	✓	✓	✓	✓	✓	✓
Cry 2A Test Line	✓				✓	
Cry 3B Test Line		✓			✓	
Cry 1F Test Line			✓		✓	
Cry 34 Test Line				✓	✓	

Please note that for ease of interpretation, the labels on each strip list the proteins in the order that their Test Lines appear, bottom (arrow end) to top (lot number end).

Kit Storage

This QuickStix Kit should be stored at room temperature (20°C), 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 Notes

- This kit is designed for screening for presence or absence only and is not meant to be quantitative.
- This product is currently not applicable for use in any other crop than corn.
- 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 and with the buffer provided in the kit. Deviation from this protocol may invalidate the results of the test.
- A negative result with this kit does not mean that the sampled tissue has not been otherwise genetically modified.
- Protect all components from hot or cold extremes of temperature when not in use. Do not leave in direct sunlight or in vehicle.
- Kit was developed with tissue samples obtained from corn in the V2 - V3 growth stage.



**For Technical Support
Contact Us At:**

EnviroLogix
500 Riverside Industrial
Parkway
Portland, ME 04103-1486
USA

Tel: (207) 797-0300
Toll Free: 866-408-4597
Fax: (207) 797-7533

e-mail:
info@envirologix.com

website:
www.envirologix.com



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