

Highlights:

- Recognizes WideStrike and Roundup Ready traits
- Results in 10 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
- 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 0.35 to 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 060 LS

Intended Use

This EnviroLogix QuickStix Combo Kit for WIDESTRIKE/RR Cotton Leaf & Seed is designed to extract and detect the presence of the traits expressed in WIDESTRIKE Insect Protection and other genetically modified cotton leaf or seed tissue. The combo strips will recognize Cry1Ac, Cry1F and CP4 EPSPS in separate regions of the same strip.

How the Test Works

Cotton plants and seeds that have been genetically modified with stacked genes express transgenic proteins in their leaf and seed tissue. To detect these 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 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 cotton leaf tissue:

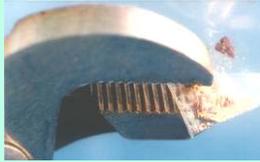
1. Sandwich a section of leaf tissue between the cap and body of the Disposable Tissue Extractor tube; snap one circular tissue punch by closing the cap. Push the leaf punch down into the tapered bottom of the tube with the pestle. Write the sample identification on the tube with a waterproof marker.
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. Add 0.35 mL Extraction Buffer.
4. Repeat the grinding step to mix tissue with water. Dispose of the pestle. Do not re-use pestles or tubes with more than one sample.

To extract cotton seed tissue:

1. Crush a single cotton 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.
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 to brown opaque color when the samples are prepared properly.



Grind Tissue



Crush single seed



Extract seed sample



Insert QuickStix

Any clearly discernable pink Test Line is considered positive

- 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

- 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 neither Cry1Ac, Cry1F, nor CP4 EPSPS protein, the strip will only show the Control Line, about 4 mm below the top pad.

Two to Four Lines – If the extract is from a sample containing Cry1Ac proteins, Cry1F proteins, and/or CP4 EPSPS (Roundup Ready) proteins, the appropriate test line(s) will appear. A Test Line for extracts containing Cry1Ac protein will appear about 4 mm below the Control Line. A Test Line for extracts containing Cry1F protein will appear about 8 mm below the Control Line. A Test Line for extracts containing CP4 EPSPS protein will appear about 12 mm below the Control Line. See interpretation guide below.



		Plant Tissue							
		Bollgard®	Bollgard / Roundup Ready	Bollgard II	Bollgard II / Roundup Ready	Bollgard II / Roundup Ready Flex	Roundup Ready	WIDESTRIKE	WIDESTRIKE / Roundup Ready
Cry1Ac Test Line								✓	✓
Cry1F Test Line								✓	✓
RR Test Line			✓		✓	✓	✓		✓
		✓ Indicates a valid positive result on specified 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 Kit can be stored at room temperature, or refrigerated for a longer shelf life. Please note the shelf life on the kit label 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 Limitations

- This kit is designed to screen 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.
- 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 interpret negative results prior to 10 minutes.
- Protect all components from hot or cold extremes of temperature when not in use. Do not leave in direct sunlight or in a vehicle.
- If green lines in the test line area interfere with interpretation when testing leaf tissue, clarify ground leaf extract by centrifuging for 30 seconds at 6500 xg.



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