

### Highlights:

- Recognizes Cry1Ac, Cry2A, Roundup Ready®, and LibertyLink® traits
- Results in 10 minutes or less

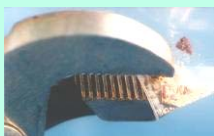
### Contents of Kit:

- 100 QuickStix Combo Strips packed in two moisture-resistant canisters
- 100 Extraction Tubes
- EB2 Extraction Buffer

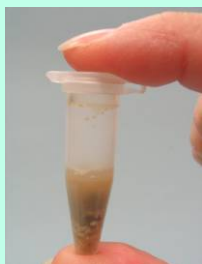
### Items Not Provided:

- Seed crusher
- Repeating pipetter or other means of dispensing 0.5 mL per well

Contact EnviroLogix to order bulk-packaged combs. Bulk kits include EB2 Extraction Buffer Concentrate. To prepare, mix 50 mL 20x Concentrate with 950 mL of distilled or deionized water per liter required. Store refrigerated when not in use; allow to come to room temperature before using.



Crush single seed



Mix with Buffer

Catalog Number AS 067 ST

## Intended Use

The EnviroLogix QuickStix Combo Kit for Multi-Trait Testing is designed to detect the presence of Cry1Ac, Cry2A, CP4 EPSPS and PAT/*bar* 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 seed. To detect these proteins with the Combo Comb Strips, 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 seed:

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.
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 cotton seed extract. The sample will travel up the strip. Use a rack to support multiple tubes if needed.



Insert Strip, read results

Any clearly discernable pink Test Line is considered positive

- 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 the Control Line, about 4 mm below the top pad.

**Two to Five Lines** – If the extract is from a sample containing Cry1Ac, Cry2A, CP4 EPSPS (Roundup Ready), and/or PAT/*bar* (LibertyLink) proteins, the appropriate test line(s) will appear. Please refer to the interpretation guide below.



|                  |  | Interpretation Guide  |   |  |   |  |  |  |  |
|------------------|--|---|---|--|---|--|--|--|--|
|                  |  | +RR, -LL, -Cry2A, -Cry 1Ac<br>(e.g. Roundup Ready, RR Flex) | +RR, -LL, -Cry2A, +Cry 1Ac<br>(e.g. Bollgard/RR or WideStrike/RR) | -RR, -LL, +Cry2A, +Cry 1Ac<br>(e.g. Bollgard II) | +RR, -LL, +Cry2A, +Cry 1Ac<br>(e.g. Bollgard II/RR) | -RR, +LL, -Cry2A, -Cry 1Ac<br>(e.g. LibertyLink) | -RR, +Cry2A, +Cry 1Ac +LL<br>(e.g. BollgardII/LibertyLink) | -RR, -LL, -Cry2A, +Cry 1Ac,<br>(e.g. Bollgard or WideStrike) | -RR, -LL, -Cry2A, -Cry 1Ac,<br>(e.g. non-GMO cotton) |
| Control Line     |  | ✓   | ✓   | ✓  | ✓   | ✓  | ✓  | ✓  | ✓  |
| Cry1Ac Test Line |  |   | ✓   | ✓  | ✓   |  | ✓  | ✓  |  |
| Cry2A Test Line  |  |   |   | ✓  | ✓   |  | ✓  |  |  |
| RR Test Line     |  | ✓   | ✓   |  | ✓   |  |  |  |  |
| LL Test Line     |  |   |   |  |   | ✓  | ✓  |  |  |

- ✓ Indicates where a test line will appear (positive result for that specific trait)
- Indicates no test line (negative result for that specific trait)

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 foil bag 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.
- This product is currently not applicable for use in any other crop than cotton.
- 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.
- 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.
- 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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