

Highlights:

- Recognizes Cry1Ac and CP4 EPSPS proteins
- 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:

- Repeating pipetter or other means of dispensing 0.5 mL

Contact EnviroLogix to order bulk-packaged kits. 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



Catalog Number AS 034 ST

Intended Use

The EnviroLogix QuickStix Combo Kit for Bollgard with Roundup Ready is designed to detect the presence of these traits at the levels typically expressed in genetically modified cotton seed. The combo strips will recognize both Cry1Ac and CP4 EPSPS proteins in separate regions of the same strip.

How the Test Works

Bollgard cotton has been genetically modified with a Bt gene to express Cry1Ac protein in their leaves and seeds. Roundup Ready cotton crops have been genetically modified to express CP4 EPSPS protein in their leaves and seeds. To detect these proteins with the QuickStix Combo 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 extraction tube or well of plate. 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.

Any clearly discernible
pink Test Line is
considered positive.



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.
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.

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

Three Lines – If the extract is from a sample containing both Cry1Ac and CP4 EPSPS proteins, a total of three lines will appear. A Test Line for extracts containing Cry1Ac protein will appear about 5 mm below the Control Line. A Test Line for extracts containing CP4 EPSPS protein will appear about 10 mm below the Control Line and approximately 5 mm below the Cry1Ac Test Line.

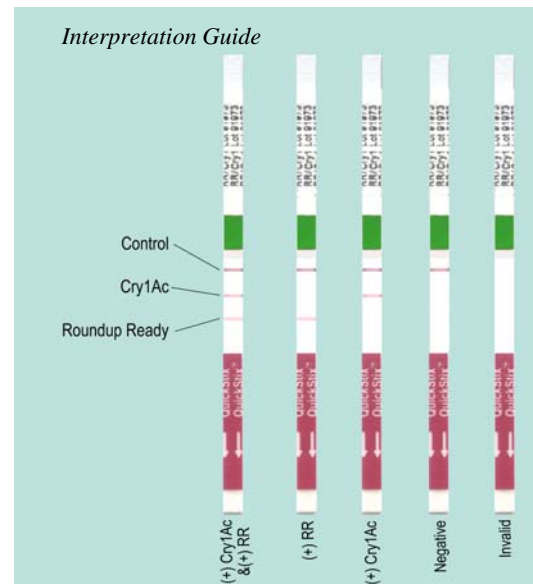
Two Lines – If the extract contains either CP4 EPSPS or Cry1Ac proteins, the strip will develop two lines. To identify the positive Test Line, compare the strip to the Interpretation Guide. Extracts containing Cry1Ac protein will exhibit a Test Line about 5 mm below the Control Line; extracts containing CP4 EPSPS protein will exhibit a Test Line about 10 mm below the Control Line.

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 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 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 5 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.



**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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