QuickStix™ Combo Kit for Multi-Trait Testing

1Ac/1F/CP4/V3 Cotton Leaf & Seed

Catalog Number AS 075 LS *Part # 11698*

Highlights:

- *Recognizes Cry1Ac, Cry1F, CP4 EPSPS & Vip3A 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)*
- *Buffer Packet (makes 1L)*

Items Not Provided:

- *Repeating pipetter or other means of dispensing 0.5 to 1.0 mL*
- *Distilled or deionized water for preparing Buffer*



Obtain Leaf Tissue



Grind Tissue

Intended Use

The EnviroLogix QuickStix Combo Kit for Multi-Trait Testing is designed to detect the presence Cry1Ac, Cry1F, and CP4 EPSPS proteins along with vegetative insecticidal proteinVip3A at levels typically expressed in genetically modified cotton seed and leaf tissue. The combo strips will recognize Cry1Ac, Cry1F and CP4 EPSPS and Vip3A 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. To detect these proteins with the EnviroLogix QuickStix Combo Strip, the sample must be extracted and the proteins solubilized in the 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

Before testing, prepare Extraction Buffer: Add contents of Buffer Packet to 1 liter of distilled or de-ionized water (room temperature) and stir to dissolve. Store in closed container and refrigerate when not in use; warm to room temperature prior to assay.

To extract cotton 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 (hint: fold leaf and snap once to get two tissue 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. Add 0.5 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 1.0 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.



Crush single seed



Extract seed sample



Insert QuickStix

Any clearly discernible pink Test Line is considered positive

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 extraction tube. 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, 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, Cry1F, CP4 EPSPS and/or Vip3A proteins, the appropriate test line(s) will appear. Please refer to the interpretation guide below.

	Interpretation Guide					
	+Vip3A, +CP4 EPSPS, +Cry1F, +Cry1Ac	-Vip3A, -CP4 EPSPS, -Cry1F, -Cry1Ac (e.g. negative for these four, or non-GMO)	+Vip3A, -CP4 EPSPS, -Cry1F, -Cry1Ab (e.g. Vip3A)	-Vip3A, -CP4 EPSPS, +Cry1F, +Cry1Ac	-Vip3A, +CP4 EPSPS, +Cry1F, +Cry1Ac	-Vip3A, +CP4 EPSPS, -Cry1F, -Cry1Ac
Control Line	✔	✔	✔	✔	✔	✔
Cry1Ac Test Line	✔			✔	✔	
Cry1F Test Line	✔			✔	✔	
CP4 Test Line	✔				✔	✔
Vip3A 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 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 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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