QuickStix™ Combo Comb Kit for Cry1A & Cry2Ae Cotton Seed Tissue

Catalog Number AS 088 STC

Highlights:

- *Recognizes Cry1A and Cry2Ae endotoxins*
- *Results in 10 minutes or less*
- *Available in convenient comb format*

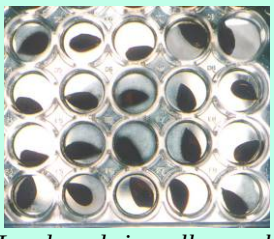
Contents of Kit:

- *48 QuickStix Combo Strips assembled as 6 combs of 8 strips packaged in a foil bag*
- *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 20X Buffer Concentrate instead of EB2. To prepare, mix the Buffer Concentrate with 950 mL of distilled or deionized water. Store refrigerated when not in use; warm to room temperature before using.



Load seeds in wells, crush

Intended Use

The EnviroLogix QuickStix Combo Comb Kit for Cry1A and Cry2Ae is designed to detect the presence of these endotoxins at the levels typically expressed in genetically modified cotton seed. The combo strips will recognize both Cry1A and Cry2Ae endotoxins in separate regions of the same strip.

How the Test Works

Crops that have been genetically modified with stacked Bt genes express Bt endotoxins in their seed. To detect these Cry1A and Cry2Ae 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 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.*

1. Load individual cotton seeds into each of the 48 wells.
2. Crush seeds with hydraulic press or equivalent.
3. The crushed state of the seed is visible through the bottom of the microplate or by gently lifting the crusher. Gently shake the crusher while lifting to dislodge any seed. Use extreme care, do not cross-contaminate the wells! **Clean crusher prongs prior to using on the next plate.**
4. Add 0.5 mL of Extraction Buffer to each well.
5. Cover plate and mix on orbital shaker or equivalent for 3 minutes. Remove and discard the plate cover.
6. Use crushed seed samples the same day they are prepared.

To improve extraction efficiency when testing cotton seed:

1. Use room temperature to lukewarm buffer to extract the seeds.
2. Longer soak times can increase the strength of the Test Line (better extraction).
3. If seed material gets stuck to the plate bottom, use the QuickStix to gently mix the extract as soon as you insert it into the well.
4. The extract takes on a yellow to brown opaque color when the seeds are crushed and mixed properly. If the extract is clear, the seed coat may be empty or the sample may not be well mixed. The seeds should contain an adequate amount of mature endosperm and embryonic tissues, not empty seed coats.

Add buffer

Remove QuickStix Combs

Insert Comb into plate

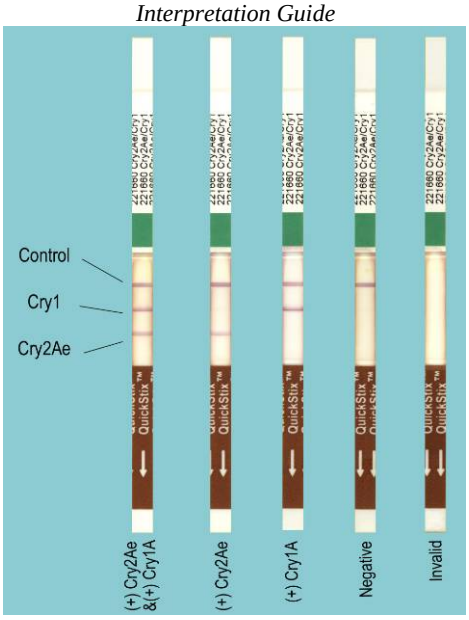
How to Run the QuickStix Comb Test

1. Allow refrigerated foil bag to come to room temperature before opening. Remove the QuickStix Combo Combs to be used. A blank space is provided to label each Comb if desired. Avoid bending the strips.
2. Place the combs into the wells with the colored tape facing you. The sample will travel up the strip.
3. Allow the strip to develop for 10 minutes before making final assay interpretations. Positive sample results may become obvious much more quickly.
4. Remove the Comb from the wells to read. To retain the strips, cut off and discard the bottom section of the strips 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.

Interpretation Guide



Any clearly discernable pink Test Line is considered positive

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 Cry2Ae and Cry1A proteins, a total of three lines will appear.

Two Lines – If the extract contains either Cry2Ae or Cry1A proteins, the strip will develop two lines. To identify the positive Test Line, compare the strip to the Interpretation Guide.

Extracts containing Cry1A protein will exhibit a Test Line about 5 mm below the Control Line; extracts containing Cry2Ae 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 box 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.
- As with all tests, it is recommended that results be confirmed with an alternate method if necessary.
- The assay has been optimized using the protocol and buffer 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.
- Warning: 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, as a weak positive sample may require the full 10 minutes for a distinct Test Line to appear.
- 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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