QuickStix™ Comb Kit for Vip3A Cotton Seed

Catalog Number AS 485 STC

Highlights:

- *Results in 10 minutes or less*
- *Strips in convenient comb format*

Contents of Kit:

- *48 QuickStix 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 EB2 Extraction Buffer Concentrate. To prepare, mix the 20X Concentrate with 950 mL of distilled or deionized water. Store refrigerated when not in use; allow to come to room temperature before using.

Cotton seeds in plate

Intended Use

The QuickStix Comb Kit for Vip3A Cotton Seed is designed to extract and detect the presence of the vegetative insecticidal protein Vip3A at the levels typically expressed in genetically modified cotton seed tissue.

How the Test Works

Cotton plants and seeds that have been genetically modified with the *vip3A(a)* gene express Vip3A protein in their seed. To detect the protein with the QuickStix Comb, the sample must first be extracted in buffer to solubilize the protein.

Each QuickStix Strip has an absorbent pad at each end. The protective tape with the arrow indicates the end of the strip to insert into the sample extract. The sample will travel up the membrane strip and be 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". Please avoid bending the strips.

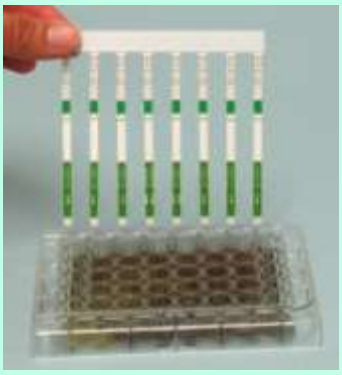
Sample Preparation

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 and cover.
5. 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:

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 Extraction Buffer



Place combs in plate

Any clearly discernable pink Test Line is considered positive

How to Run the QuickStix Comb Test

1. Allow refrigerated foil bag to come to room temperature before opening. Remove a QuickStix Comb from the bag and reseal the bag. A blank space is provided to label each Comb if desired. Place the comb of strips into the plate wells, being sure to insert the end indicated by the arrows on the protective tape.

2. After inserting the comb into the extracts, liquid will travel up the membrane strips toward the absorbent pads at the top of the strips. Soon after complete wetting of the membrane, a line will appear on the membrane approximately 1/4 inch below the top absorbent pad. This is the Control Line.

3. The results should develop within 5 to 10 minutes. Allow the strips to develop for a full 10 minutes before making final negative assay interpretations. Strongly positive samples may show results much sooner. Remove the QuickStix Comb from the wells to read. To retain the sticks, cut off and discard the bottom section of each strip covered by the arrow tape.

Interpreting the Results

Development of the Control Line within ten 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.

If the extract is from a *vip3A(a)*-modified seed, a second line (Test Line) will develop on the membrane strip between the Control Line and the protective tape. *The results should be interpreted as positive for Vip3A protein expression.*

If no Test Line is observed after 10 minutes have elapsed, the results should be interpreted as negative. A negative result means the sample contains less Vip3A protein than is typically expressed in the seeds of *vip3A(a)*-modified cotton plants.

Kit Storage

This Kit should be stored at room temperature, or refrigerated for 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. Important: do not open the foil bag until you are ready to use the combs. Allow foil bags to come to room temperature before opening to prevent condensation. Immediately re-seal unused QuickStix Combs in the bag.

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.

- 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 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.

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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