QuickStix™ AP Kit for Cry2A Cottonseed

Catalog Number AS 005 AP

Highlights:

- *Detects 1 in 400*
- *Results in 10 minutes or less*
- *Available as 100-strip kits or bulk packaging*

Contents of Kit:

- *100 QuickStix Strips packed in two moisture-resistant canisters*
- *100 transfer pipettes*
- *100 reaction vials*
- *Buffer Concentrate*

Items Not Provided:

- *Waring blender, model 31BL91 or equivalent**
- *Glass jar adapter (Eberbach # E8495)*
- *Glass Mason jars*
- *Protective cover for blender jar while grinding*
- *Graduated cylinder*

Intended Use

The EnviroLogix QuickStix AP Kit for Cry2A Cottonseed is designed to extract and detect the adventitious presence of the Cry2A proteins at the levels typically expressed in genetically modified cottonseed. The detection level of the QuickStix AP Kit for Cry2A is 0.25% based on tests conducted with Bollgard II® cottonseed (i.e. one positive seed in 400 conventional cottonseeds). For Cry2A detection in cotton leaf tissue or individual seeds, please use QuickStix Cat# AS 005 LS.

NOTE: A negative result with this test on cottonseed extracts does not necessarily rule out the presence of genetically modified material in the sample.

How the Test Works

In order to detect the Cry2A proteins with this Kit, the sample must first be ground and 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 reaction vial. 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

Prepare 1X Extraction Buffer as directed on the Buffer Concentrate container.

1. Determine the number of seeds to be tested – the table below lists the guidelines for jar size and grinding time according to sample size.

***NOTE:** If using a different grinding method than the Waring blender, the buffer volume may need to be adjusted. Please contact Technical Service (1-866-408-4597) for details.

Commodity	Sample Size	Jar Size (oz.)	Grind Time (sec.)	Buffer Volume (mL)*
Cottonseed	400 seeds	8	20	140 ±5

2. Choose the appropriate size glass Mason-type jar for sample size and count the seeds into it.
3. Put protective cover over the jar attached to the blender.
4. Grind sample with a Waring blender (or equivalent) and jar adapter on high speed for 20 seconds or until all whole grains are broken.
5. Add the volume of 1X Extraction Buffer called for in the table. For convenience, buffer added to the bottom glass rim of the jar is sufficient to extract the sample.
6. Cap the jar and shake vigorously for 10 seconds to thoroughly wet all of the cottonseed in the sample. Allow sample to settle for about one minute before

drawing off liquid. Best results are achieved when samples are tested immediately after extraction.

7. Draw up enough liquid portion from above the settled sample to fill the long narrow tip of the transfer pipette up to the line at the top of the flared portion of the pipette bulb. Avoid pulling up particles. Dispense extract into reaction vial.

8. To prevent cross-contamination, thoroughly clean blender parts and jars to remove dust and residue prior to preparation of a second sample. Use a new transfer pipette and reaction vial for each sample.

Avoid pulling up particles when drawing sample

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 reaction vial. The sample will travel up the strip. Reaction vials will stand on their own or may be inserted into the cardboard rack provided.

3. Allow the strip to develop for 10 minutes before making final assay interpretations. Positive sample results may become obvious much more quickly.

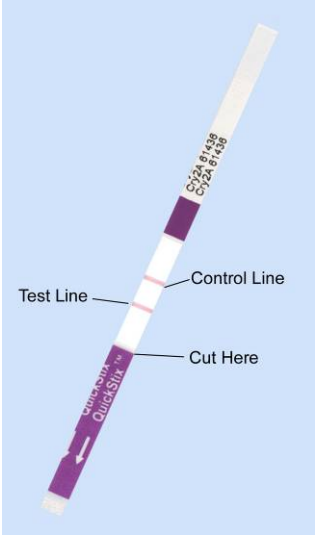
4. To retain the strip, cut off and discard the bottom section of the strip covered by the arrow tape.

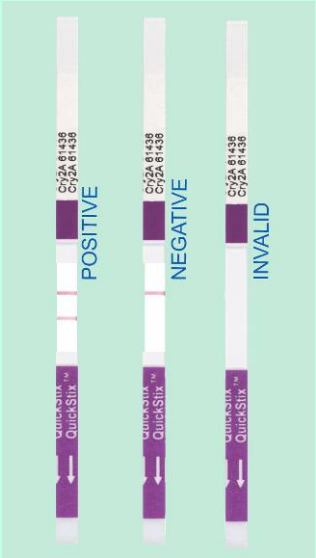
Interpreting the Results

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.

If the sample extract contains Cry2A, a second line (Test Line) will develop on the membrane strip between the Control Line and the protective arrow tape. The results should be interpreted as positive for Cry2A expression.

If no Test Line is observed after 10 minutes, the results should be interpreted as negative. A negative result means the sample contains less than 0.25% of Cry2A.





Any clearly discernable pink Test Line is considered positive

Kit Storage

QuickStix strips can be stored at room temperature, or refrigerated for a longer shelf life. 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 desiccated canister until ready to use the test strips.

Precautions and Notes

- This kit is designed to screen for presence or absence only, and is not meant to be quantitative.
- This product is currently not applicable for use in any other crop or in leaf or individual seed testing.

- As with all tests, it is recommended that results be confirmed by an alternate method if 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 diagnostic tool 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.
- Warning: a strong positive result may safely be interpreted in as little as 2 minutes after sample addition. It is not safe to interpret weak positive or negative results prior to 10 minutes.
- The assay has been optimized to develop an easily discernable red line at 1 in 400; experienced users may detect faint lines in samples with even lower concentrations.
- Centrifuging samples briefly (30 seconds at 6000xg or more) may clarify samples. Pelleting fine particulates and separating oils from the aqueous portion containing detectable proteins can improve sample flow, reduce background, and aid interpretation.
- DO NOT leave in direct sunlight or in vehicle. Protect all components from hot or cold extremes of temperature when not in use.

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