QuickStix™ Combo Comb Kit for Bollgard II®/ Roundup Ready® Cotton Seed

Highlights:

- *Recognizes Cry1Ac, Cry2A, and Roundup Ready traits*
- *Results in 10 minutes or less*
- *Strips in convenient comb format*

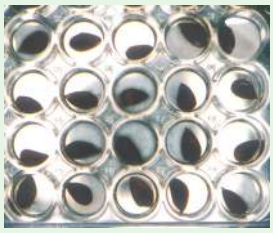
Contents of Kit:

- *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 0.5-0.6 mL per well (delinted) and/or 0.25 and 0.5 mL per well (fuzzy)*
- *Pin plate or other means of stirring extract (fuzzy)*

Contact EnviroLogix to order bulk-packaged combs. 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.



Load seeds in wells, crush

Catalog Number AS 046 STC

Intended Use

The EnviroLogix QuickStix Combo Comb Kit for Bollgard II/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 Cry1Ac, Cry2A and CP4 EPSPS 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 seed. To detect these 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 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

Note: *If Extraction Buffer has been refrigerated, allow it to warm up to room temperature before preparing samples.*

A. Delinted Seed:

A1. Load individual delinted cotton seeds into each of the 48 wells.

A2. Crush seeds with hydraulic press or equivalent.

A3. 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.**

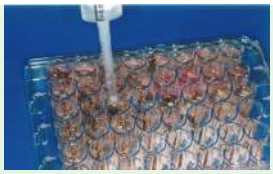
A4. Add 0.5 mL of Extraction Buffer to each well and cover.

A5. Mix on orbital shaker or equivalent for 3 minutes. Remove and discard the plate cover.

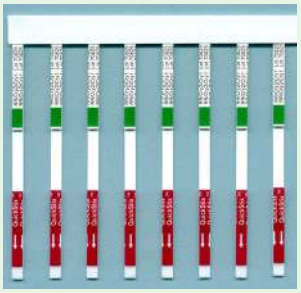
A6. Use crushed seed samples the same day they are prepared.

To improve extraction efficiency:

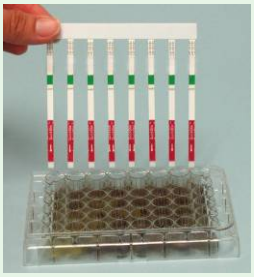
- Use room temperature to lukewarm buffer to extract the seeds.
- Longer soak times can increase the strength of the Test Line (better extraction).
- If seed material gets stuck to the plate bottom, use the QuickStix to gently mix the extract as soon as you insert it into the well.
- The extract takes 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



Add Extraction Buffer



Remove QuickStix Combs



Insert Combs into plate

Any clearly discernable pink Test Line is considered positive

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.

B. Fuzzy Seed:

B1. Add 0.25 mL of Extraction Buffer to each well.

B2. Remove any extra linters on the fuzzy cotton seeds and load one seed into each of the 48 wells.

B3. Crush seeds twice with hydraulic press (4-5 ton) or equivalent.

B4. 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.**

B5. Add 0.5 mL of Extraction Buffer to each well and cover.

B6. Mix covered plate on orbital shaker (~600 rpm) or equivalent for 10 minutes. Remove and discard the plate cover.

B7. Use pin plate or equivalent to vigorously break up seeds and mix with extract until all wells exhibit cloudy extract. Use extreme care not to cross-contaminate the wells. **Clean pin plate prior to using on next plate.**

B8. Use crushed seed samples the same day they are prepared.

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 (if testing delinted seed) or 7 minutes (for fuzzy seed) 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 the test time 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 neither Cry1Ac, Cry2A, nor CP4 EPSPS protein, the strip will only show the Control Line, about 4 mm below the top pad.

Two to Four Lines – If the extract is from a sample containing Cry1Ac proteins, Cry2A proteins, and/or CP4 EPSPS (Roundup Ready) proteins, the appropriate test line(s) will appear. A Test Line for extracts containing Cry1Ac protein will appear about 4 mm below the Control Line. A Test Line for extracts containing Cry2A protein will appear about 8 mm below the Control Line. A Test Line for extracts containing CP4 EPSPS protein will appear about 12 mm below the Control Line. See interpretation guide below.

	Interpretation Guide							
	+RR, +Cry2A, +Cry 1Ac	-RR, +Cry2A, +Cry1Ac	+RR, -Cry2A, +Cry1Ac	+RR, +Cry2A, -Cry1Ac	-RR, -Cry2A, +Cry1Ac	+RR, -Cry2A, -Cry1Ac	-RR, +Cry2A, -Cry1Ac	-RR, -Cry2A, -Cry1Ac
Control Line	—	—	—	—	—	—	—	—
Cry1Ac Test Line	—	—	—		—			
Cry2A Test Line	—	—		—			—	
RR Test Line	—		—	—		—		

— indicates where a line will appear on the Strip

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).

Cross-reactivity

The following materials have been tested with this kit and have been found to cause no false positive results:

- GMO Materials as listed in Table 1 below.

Table 1

	Seed Tissue										Proteins				
	Bollgard®	Bollgard / Roundup Ready	Bollgard II	Bollgard II / Roundup Ready	Bollgard II / Roundup Ready Flex	Cry2A	Roundup Ready	LibertyLink® PAT/*bar*	WideStrike™	WideStrike / Roundup Ready	Cry1Ab	Cry1Ac	CP4 EPSPS	PAT from *pat* gene	PAT from *bar* gene
Cry1Ac Test Line	✔	✔	✔	✔	✔				✔	✔	✔	✔			
Cry2A Test Line			✔	✔	✔	✔									
RR Test Line		✔		✔	✔		✔			✔			✔		
	from other GMO cotton events										*tested at concentrations at least four times higher than reported in commercial crops*				

✔ *Indicates a valid positive result on specified test line*

Indicates a negative result on specified test 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 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.
- 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 test time 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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