

# QuickStix™ Combo Kit for Cry1Ab & Cry3Bb Corn Leaf & Seed

### Highlights:

- Recognizes Cry1Ab and Cry3Bb proteins
- Available as 100-strip individual kits, or in bulk packaging

### Contents of Kit:

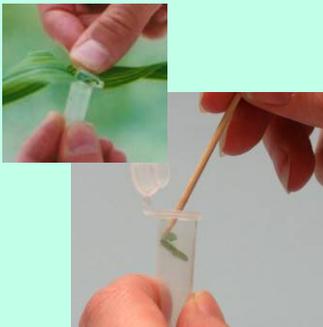
- 100 QuickStix Strips packed in two moisture-resistant canisters
- EB2 Extraction Buffer
- Dropper bottle
- 100 Disposable Tissue Extractor Tubes (tapered snap-cap vials)

### Items Not Provided:

- Toothpicks, or equivalent
- Plastic bags

Contact EnviroLogix to order bulk-packaged kits. Bulk kits include EB2 Extraction Buffer Concentrate. To prepare 1 liter, mix 50 mL of 20x Concentrate with 950 mL of distilled or deionized water. Store refrigerated when not in use; allow to come to room temperature before using. Expiration date for prepared buffer is equal to that stated on the 20x container.

### Leaf tissue testing:



Obtain leaf tissue, push down

Catalog Number AS 038 LS

## Intended Use

The EnviroLogix QuickStix Combo Kit for Cry1Ab & Cry3Bb is a qualitative test designed to detect the presence of Cry1Ab and Cry3Bb proteins at the levels typically expressed in genetically modified corn leaf or seed tissue of YieldGard® Plus, YieldGard VT Rootworm/RR2™, and YieldGard VT Triple™ corn hybrids. This kit will recognize both proteins in separate regions of the strip as follows:

		YieldGard Rootworm	YieldGard Plus YieldGard Plus/RR2	YieldGard VT Rootworm/RR2	YieldGard VT Triple YieldGard/RR2
Cry3Bb	•	•	•	•	
Cry1Ab		•		•	•

## How the Test Works

In order to test for the Cry1Ab and Cry3Bb proteins produced in these corn hybrids, the tissue samples must be extracted with Extraction Buffer.

Each QuickStix strip has an absorbent pad at each end. The protective tape with arrows indicates the end of the strip to insert into the sample extract. 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

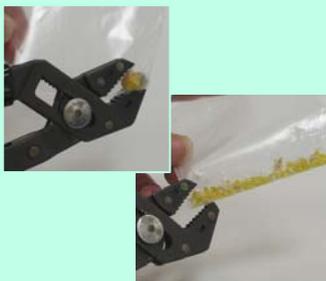
**Note:** If Extraction Buffer has been refrigerated, allow to warm up to room temperature before preparing samples. Fill the dropper bottle provided with Extraction Buffer.

### To extract corn leaf tissue:

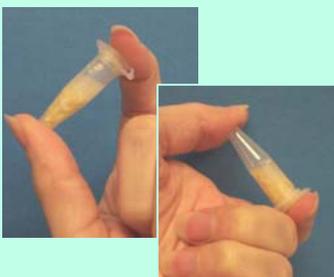
1. Use *extreme* caution to prevent sample-to-sample cross-contamination with plant tissues, fluids, or disposables. Repeat the protocol for each sample to be tested using a new tube and toothpick. Avoid touching the pad at the bottom of the strip after handling leaf tissue.
2. Sandwich a section of leaf tissue between the cap and body of the Disposable Tissue Extractor tube. Snap **two** circular tissue punches by closing the cap.
3. Use a toothpick or similar device to push the leaf punches down into the tube. Write the sample identification on the tube with a waterproof marker. Discard toothpick.



Add Buffer, then shake

**Seed tissue testing:**

Crush seed

Extract seed sample—  
vigorous shaking is vital

Insert QuickStix

4. Holding the dropper bottle vertically, add **10 drops** (~0.5 mL) of Extraction Buffer into the tube containing the leaf punches. Do not touch the dropper tip to the tube or leaf sample.
5. Close the tube cap securely. Shake the tube vigorously for 30 seconds using an up-and-down motion. Tap the solid materials to the bottom of the tube.

**To extract corn seed tissue:**

1. Use *extreme* caution to prevent sample-to-sample cross-contamination with plant tissues, fluids, or disposables. Repeat the protocol for each sample to be tested using a new plastic bag and tube for each.
2. Finely crush a single corn seed (*Suggestion: use pliers with seed in resealable plastic bag*). Transfer to a tissue extraction tube marked with sample identification.
3. Holding the dropper bottle vertically, carefully dispense **20 drops** (~1.0 mL) of Extraction Buffer into the tube containing crushed seed. Do not touch the dropper tip to the tube or seed sample.
4. Close the tube cap securely. Shake the tube vigorously for **30 to 60 seconds** using an up-and-down, end-to-end motion, ensuring that all solids (including those in the tapered end of the tube) are wet and forcibly very well mixed throughout the buffer. **This extensive, vigorous shaking will improve extraction and signal intensity.** After shaking step, tap the solid materials to the bottom of the tube. The extract should appear very cloudy.

**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. If testing **leaf tissue**, allow the strip to develop in the extract for 5 minutes before making final assay interpretations. If testing **seed tissue**, allow the strip to develop in the extract for 10 minutes before making final 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 5 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 neither Cry1Ab nor Cry3Bb protein, the strip will only show the Control Line.

**Two Lines** – If the extract contains either Cry1Ab or Cry3Bb proteins, the strip will develop two lines. To identify the positive Test Line, compare the strip to the Interpretation Guide. Extracts containing Cry3Bb protein will exhibit a Test Line about 5 mm below the Control Line; extracts containing Cry1Ab protein will exhibit a Test Line about 10 mm below the Control Line.

**Three Lines** – If the extract is from a sample containing both Cry1Ab and Cry3Bb proteins, a total of three lines will appear. A Test Line for extracts containing Cry3Bb protein will appear about 5 mm below the Control Line. A Test Line for extracts containing Cry1Ab protein will appear about 10 mm below the Control Line and approximately 5 mm below the Cry3Bb Test Line.



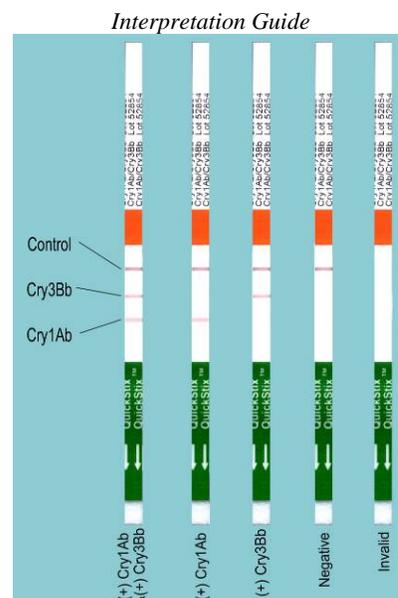
Refer to the table on page 1 for the names of the varieties containing Cry1Ab and Cry3Bb.

## 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 (see “Precautions and Notes”). 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 corn.
- 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.
- Protect all components from hot or cold extremes of temperature when not in use. Prolonged exposure to high temperatures may adversely affect the test results. Do not leave in direct sunlight or in vehicle.
- 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 seed lot from which the leaf or seed 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 seed 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.
- Age and condition of leaf tissue sampled can impact the intensity of the result.
- Corn seed should be finely crushed and thoroughly mixed with buffer by extensive, vigorous end-to-end shaking to achieve maximum signal intensity.
- 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 the full assay time has elapsed. A weakly positive single seed sample may require a full 10 minutes for a distinct Test Line to appear.
- Use extreme caution to prevent sample-to-sample cross-contamination with tissues, fluids, or disposables. Avoid touching the pad at the bottom of the strip after handling leaf tissue. Discard disposables after one use.
- This kit will detect Cry3Bb protein found in multiple corn hybrids including YieldGard Rootworm, YieldGard Plus, YieldGard VT Rootworm/RR2, and YieldGard VT Triple. The test does not necessarily distinguish between these corn hybrids.



*Any clearly discernable pink Test Line is considered positive*



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