

Highlights:

- Positive results in 5 minutes or less
- 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 Extractors (each consisting of a tube and pestle, with punch cap)

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, grind

Catalog Number AS 015 LS

Intended Use

The EnviroLogix QuickStix Kit for Cry3Bb Corn Leaf & Seed is a qualitative test designed to extract and detect the presence of Cry3Bb protein in leaf and seed tissue extracts of YieldGard® Rootworm, YieldGard® Plus, YieldGard VT Rootworm/RR2™, and YieldGard VT Triple™ corn hybrids.

How the Test Works

In order to test for the Cry3Bb protein 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 it 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 pestle. 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 **one** circular punch by closing the cap.
3. Insert the pestle into the tube, pushing leaf tissue down into the tapered bottom, and grind the tissue by rotating the pestle against the sides of the tube with twisting motions. Continue this process for 20 to 30 seconds, or until the leaf tissue is well ground.
4. Holding the dropper bottle vertically, add **10 drops** (~0.5 mL) of Extraction Buffer into the tube containing the leaf punch. Do not touch the dropper tip to the tube or leaf sample.
5. Repeat the grinding step to mix tissue with Extraction Buffer. Tap the solid materials to the bottom of the tube. Dispose of the pestle (do not re-use pestles on more than one sample).

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.

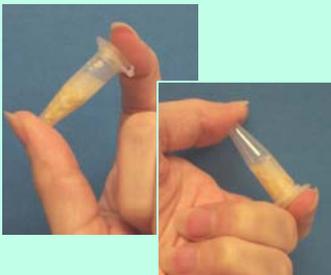


Add buffer, grind again

Seed tissue testing:



Crush seed



Extract seed sample—
vigorous shaking is vital



Insert QuickStix

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. Allow the strip to develop in the extract for 5 minutes before making final assay interpretations. Positive sample results may become obvious much more quickly. Allow the test to run for the full 5 minutes before concluding a sample is negative.
4. To retain the strip, cut off and discard the bottom section of the strip covered by the arrow tape.

Interpreting the Results

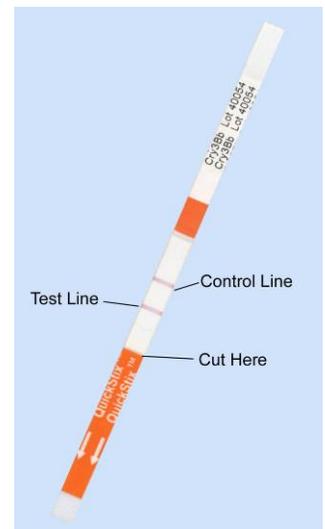
Development of the Control Line within 5 minutes indicates that the strip has functioned properly. Any strip that does not develop a Control Line should be discarded and the sample re-tested using another strip.

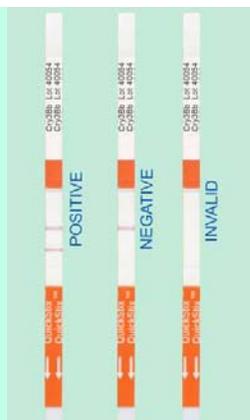
If the sample extract contains Cry3Bb protein, a second line (Test Line) will develop on the membrane strip between the Control Line and the protective tape within 5 minutes of sample addition. *The results should be interpreted as positive for Cry3Bb protein expression.*

If the extract is from a negative sample, the strip will only develop 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 (see “Precautions and Notes”). Do not open the desiccated canister until ready to use the test strips.





Any clearly discernable pink Test Line is considered positive



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 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 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 be safely 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 5 minutes have elapsed.
- 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.



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