

### Highlights:

- Results in 5 minutes or less
- Available in 100-strip individual kit format or bulk packaging

### Contents of Kit:

- 100 QuickStix Strips packed in two moisture-resistant canisters
- 100 Disposable Tissue Extractors, each consisting of a tube with punch cap and pestle (optional item with bulk packaging)
- EB2 Extraction buffer
- 1 dropper bottle for dispensing buffer

Contact EnviroLogix to order bulk-packaged kits. 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.



Obtain Leaf Tissue

Catalog Number AS 054 LS

## Intended Use

The EnviroLogix QuickStix Kit for HERCULEX™ RW screens for the presence of Bt Cry34Ab1 in HERCULEX™ RW **Corn Leaf and Corn Seed** tissues. It is not currently intended for use with any other crops. For Cry34Ab1 detection in corn bulk grain, please use QuickStix Cat# AS 054 BG.

## How the Test Works

To detect the Cry34Ab1 protein with the EnviroLogix QuickStix Strip, tissue samples must be extracted and the protein solubilized in EB2 extraction buffer.

Each QuickStix Strip has an absorbent pad at each end. The protective tape with the arrow indicates which end of the strip to insert into the extraction tube. 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

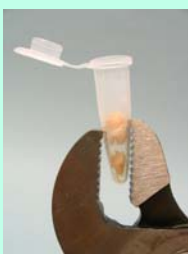
Prior to beginning, fill the dropper bottle provided with extraction buffer. If extraction buffer has been refrigerated, allow it to warm to room temperature before preparing samples.

### To extract corn leaf tissue:

1. Sandwich a section of leaf tissue between the cap and body of the Disposable Tissue Extractor tube. Snap **one** circular tissue punch by closing the cap. Push the leaf punch down into the tapered bottom of the tube with the pestle. Write the sample identification on the tube with a waterproof marker.
2. Insert the pestle into the tube 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.
3. Holding the dropper bottle vertically, carefully add 10 drops of buffer into each tube containing corn leaf tissue. If using a pipette, add 0.5 mL of buffer.
4. Repeat the grinding step to mix tissue with buffer. Dispose of the pestle. Do not re-use pestles on more than one sample.
5. Optional: At average expression levels of Bt Cry34Ab1 in leaf tissue, it is not necessary to grind the leaf punch to detect the protein. Simply add the buffer to the leaf punch, close the cap, shake the tube a few times and add the strip.

### To extract corn seed:

1. Crush a single corn seed (*Suggestion: use pliers with seed in microcentrifuge tube or resealable plastic bag*). Transfer to a tissue extraction tube marked with sample identification. Note: Complete crushing of seed improves extraction efficiency and test performance.
2. Holding the dropper bottle vertically, add 20 drops of buffer into the tube. If using a pipette, add 1 mL of buffer.

*Grind Tissue**Crush Seeds**Extract Seed Sample**Insert QuickStix Strip*

- Close the tube cap securely. Shake the tube vigorously for 20 to 30 seconds. Allow the solid material to settle to the bottom of the tube.
- Repeat the protocol for each sample to be tested, using a new tube for each. Use caution to prevent sample-to-sample cross-contamination with plant tissue, fluids, crushing equipment (*pliers*) or disposables.

## How to Run the QuickStix Strip Test

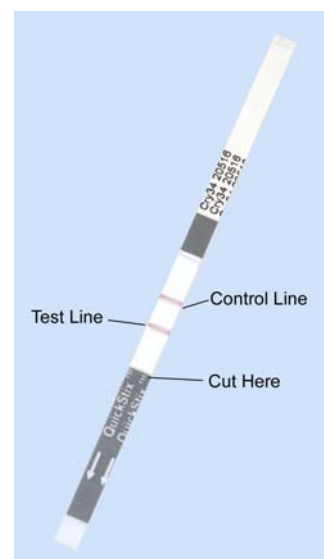
- Allow refrigerated canisters to come to room temperature before opening. Remove the QuickStix Strips to be used. Avoid bending the strips. Reseal the canister immediately.
- Place the strip into the extraction tube. The sample will travel up the strip. Use a rack to support multiple tubes if needed.
- Allow the strip to develop for 5 minutes before making final assay interpretations. Positive sample results may become obvious much more quickly.
- If you wish 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 the Bt Cry34Ab1 protein, 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 Bt Cry34Ab1 protein expression.

If no Test Line is observed after 5 minutes, the results should be interpreted as negative. A negative result means the sample contains less Bt Cry34Ab1 than is typically expressed in the tissues of corn plants.



## Kit Storage

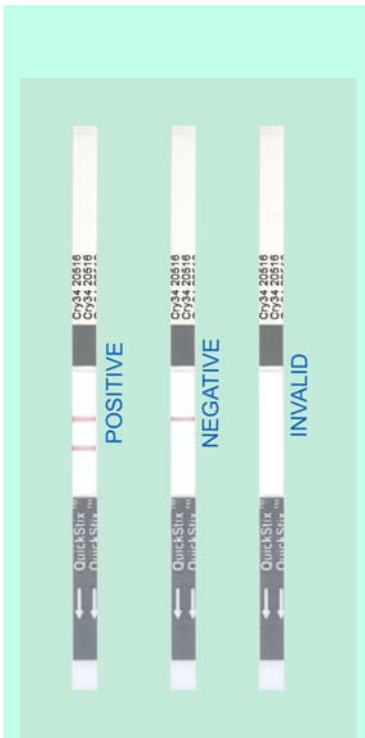
This QuickStix Kit can be stored at room temperature, or refrigerated for a longer shelf life. Note the shelf life on the kit box for each storage temperature. The kit may be used in field applications; however, prolonged exposure to high temperatures may adversely affect the test results. Do not open the desiccated canister until ready to use the test strips.

## Cross-reactivity

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

Proteins: Bt Cry1Ab, Bt Cry1Ac, Bt Cry2Ab, Bt Cry3Bb, CP4EPSPS, PAT/*pat*, and PAT/*bar*.

Other GMO Events in corn leaf and seed: YieldGard® (Bt 11, MON 810), LibertyLink®, Starlink®, Roundup Ready® (GA21, NK603) and Herculex™ I.



*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.
- 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 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 5 minutes has elapsed. A weakly positive sample may require the full 5 minutes for a distinct Test Line to appear.
- 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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## License

EnviroLogix has developed this kit using proprietary reagents as well as reagents licensed from Pioneer (DuPont Agriculture & Nutrition) Company and Dow AgroSciences.

This test kit has been validated and approved by Dow AgroSciences for detection of the HERCULEX™ RW trait.

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