

### Highlights:

- Results in 5 minutes or less
- Available as 100-strip individual kits, or bulk-packaged strips

### 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  
(two punches)

Catalog Number AS 014 LS

## Intended Use

The EnviroLogix QuickStix Kit for LibertyLink is designed to extract and detect the PAT/*pat* protein at levels typically expressed in LibertyLink corn or soybean leaf and seed tissue. The PAT/*pat* protein can be found in T25 corn, Herculex™ I, and YieldGard® Corn Borer (Bt11) corn. For PAT/*pat* detection in corn bulk grain, please use QuickStix Cat. No. AQ 014 BG; for PAT/*pat* detection in bulk soybeans, use Cat. No. AQ 014 BGB.

## How the Test Works

Corn and soybean crops that have been genetically modified with a *pat* gene express PAT/*pat* protein in their tissue. To detect the protein with the QuickStix Strip, tissue samples must be extracted and the protein solubilized in the Extraction Buffer provided.

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 extraction tube. 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.”

## Sample Preparation

### Corn or soy leaf tissue:

1. 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. Push the leaf punches down into the tapered bottom of the tube with the pestle. Sample identification should be marked 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. Uncap the bottle of Extraction Buffer and invert it directly over the Tissue Extractor tube. Carefully squeeze 5 drops (0.25 mL) of Buffer into the tube containing leaf.
4. Repeat the grinding step to mix tissue with Extraction Buffer. Dispose of the pestle (do not re-use pestles on more than one sample).

### Corn seed:

1. Crush a single corn kernel (*Suggestion: Use pliers with seed in resealable bag for hard kernels*). Transfer to a Disposable Tissue Extractor tube marked with sample identification.
2. Uncap the bottle of Extraction Buffer and invert it directly over the Tissue Extractor tube. Carefully squeeze 10 drops (0.5 mL) of Buffer into the tube. Alternatively, remove the dropper tip from the bottle, or use prepared Extraction Buffer made from Concentrate, and pipette 0.5 mL (500 microliters) into the tube.



Grind tissue, add Buffer

**Seed tissue testing:**

Crush single seed



Extract seed sample



Insert QuickStix

- Close the tube cap securely and 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 and pestle for each. Use caution to prevent sample-to-sample cross-contamination with plant tissue, fluids, 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 Tissue Extractor 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.
- To retain the strip, cut off and discard the bottom section of the strip covered by the arrow tape.

## Interpreting the Results

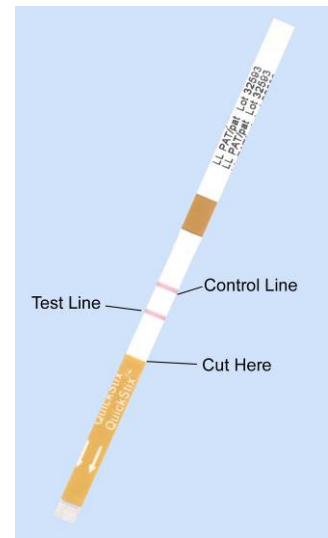
Development of the Control Line within 5 minutes indicates that the strip has functioned properly. Any strip that does not develop a Control Line should be discarded and the sample re-tested using another strip.

If the sample extract contained PAT/*pat* 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 PAT/*pat* protein expression. Any clearly discernible pink Test Line is considered positive.*

If no Test Line is observed after 5 minutes have elapsed, the results should be interpreted as negative, meaning that the sample contained less PAT/*pat* protein than is typically expressed in the tissues of LibertyLink-modified plants.

**Warning:** A negative result with this test on seed or leaf extracts does not necessarily rule out the presence of genetically modified material in the sample.

Note: This test **will not** detect PAT/*bar* proteins as expressed in StarLink corn and LibertyLink PAT/*bar* canola or cotton. This test is capable of detecting PAT/*pat* proteins such as those found in LibertyLink (PAT/*pat*) corn and soybean; YieldGard (Bt11 & PAT/*pat*) and Herculex I (Cry1F) corn; and LibertyLink (PAT/*pat*) canola. However, because of much lower expression levels in YieldGard and Herculex corn, the test lines will appear lighter than those appearing when testing LibertyLink corn or soybean samples.



## Kit Storage

QuickStix 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.



*Any 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 with an alternate method if necessary.
- The assay has been optimized using the protocol and buffer 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.
- Warning: 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, as a weak 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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