

### Contents of Kit:

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

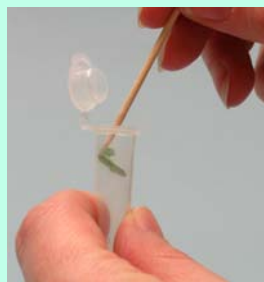
### Items Not Provided:

- Toothpicks, or equivalent

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.



Obtain Leaf Tissue



Push Leaf Tissue Down

Catalog Number AS 045 LT

## Intended Use

The EnviroLogix QuickStix Kit for Roundup Ready Alfalfa Leaf is a qualitative test designed to extract and detect the presence of CP4 EPSPS protein in alfalfa leaf tissue.

## How the Test Works

In order to test for the Roundup Ready protein expressed by Roundup Ready alfalfa leaf, the unground leaf 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: A field can be classified as being positive for Roundup Ready with 95% confidence that the true percentage of the field that is RR is greater than 20%, with an analysis of only 4-6 samples (using statistical analysis such as SeedCalc7).*

*Divide the field into equal quadrants and sample one plant leaf from each section. Follow test procedure below. If three or more of the samples are negative, the field is conventional alfalfa (at the 95% confidence level). If three of the four samples are positive, the field is Roundup Ready alfalfa. If two of the four are positive, randomly sample two additional locations within the field. If these additional samples are positive, the field is Roundup Ready alfalfa.*

1. Sandwich a single leaflet between the cap and body of the Disposable Tissue Extractor tube. Snap one circular punch by closing the cap.
2. Use a toothpick or similar device, to push the leaf punch down into the tube. Write the sample identification on the tube with a waterproof marker. Discard the toothpick. **Do not grind the tissue.**
3. 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.
4. Close the tube cap securely. Shake the tube vigorously for 20 – 30 seconds using an up-and-down motion. Tap the solid materials to the bottom of the tube.

## 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.



Add Buffer, then Shake



Insert QuickStix



Any clearly discernable pink Test Line is considered positive

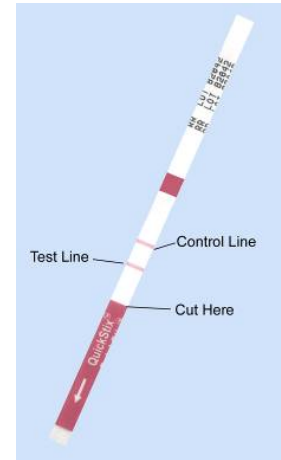
2. Place the strip into the extraction tube. The sample will travel up the strip. Use a rack to support multiple tubes if needed.
3. Allow the strip to develop for 5 minutes before making final assay interpretations. Positive sample results may become obvious much more quickly.
4. To retain the strip, cut off and discard the bottom section of the strip covered by the arrow tape.

**NOTE:** Use extreme caution to prevent sample-to-sample cross-contamination with tissues, fluids, or disposables. Discard disposables after one use.

## 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 Roundup Ready CP4 EPSPS protein, a second line (Test Line) will develop on the membrane strip between the Control Line and the protective tape. *The results should be interpreted as positive for CP4 EPSPS 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 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 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.
- As with all tests, it is recommended that results be confirmed by an alternate method when necessary.
- The assay has been optimized with the protocol and with the 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 diagnostic tool reflect the condition of the working sample directly tested. Extrapolation as to the condition of the planted seed lot from which the leaf sample extract was derived should be based on sound sampling procedures and statistical calculations which address the following: random sampling effects, non-random 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.
- 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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