

Contents of Kit:

- 50 QuickStix Strips packed in a moisture-resistant canister
- 50 disposable sample cups with lids
- EB2 Extraction Buffer
- reaction vials
- transfer pipettes
- measuring cup for buffer

Items Not Provided:

- Portable scale to weigh hay (ProScale CP-120 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.



Catalog Number AS 045 AH

Intended Use

This EnviroLogix QuickStix Kit for CP4 EPSPS Alfalfa Hay is designed to extract and detect the presence of CP4 EPSPS protein in cored or ground alfalfa hay at levels of approximately 5% or above, dependent upon the expression level of the Roundup Ready plant.

This test is intended to give the producer, purchaser, or exporter a screening method for identification of the presence of Roundup Ready alfalfa hay from cored field samples and/or ground hay lab samples at levels of 5% or more, based on weight/weight (w/w) ratios. The results of each sample tested are only as representative as the sample is of the entire lot, so adherence to proper principles of hay sampling is key.

How the Test Works

In order to test for the CP4 EPSPS protein expressed by Roundup Ready alfalfa hay, an extract of the composite hay sample must be prepared. Extracts of cored hay and ground hay are prepared for testing using different methods (see Sample Preparation section).

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

Important-Representative Sample: It is very important that the sample tested is **representative of the entire lot of hay**. A composite sample for each hay lot is required to run the test. A helpful reference is "[Sampling Considerations for Detection of Genetically Engineered \(GE\) Traits in Alfalfa Hay](#)" by Dan Putnam, University of California, Davis. A composite sample for each hay lot is required to run the test.

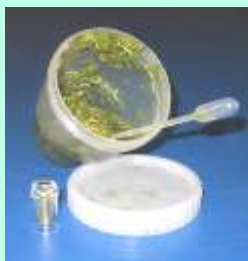
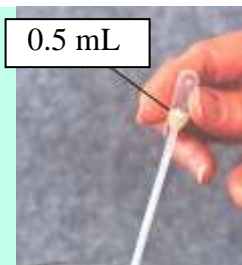
1. Select alfalfa hay sample type for testing below. **Note change in EB2 Extraction Buffer volume dependent upon sample type.**

A. TEST of CORED HAY - Weigh 5 grams from the mixed, composite sample of cores and place in the disposable sample cup provided with the kit.

Using the measuring cup, **add 40 mL** of Extraction Buffer to the disposable sample cup containing hay, and securely close the lid.

B. TEST of GROUND HAY - After grinding composite sample, remove 1 gram and place in the disposable sample cup provided in the kit. Use a balance to weigh out ground material carefully and avoid cross-contaminating samples with hay fines.

Using the measuring cup, **add 80 mL** of Extraction Buffer to the disposable sample cup containing hay, and securely close the lid.



*This end in sample—
Make sure extract level
is no higher than
the tops of the arrows*

- Shake cup for 1 minute by hand. Look at the bottom of the cup and confirm that all material is wet from the shaking process before proceeding. Continue shaking until all material is wet.
- Draw up enough liquid portion from the settled sample to fill the long narrow tip of the transfer pipette up to the line at the top of the flared portion of the pipette bulb (see illustration, left). Avoid pulling up particles. Dispense extract (~0.5 mL) into reaction vial.

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 one strip, colored arrows pointing down, into the reaction vial containing extract. The sample will travel up the strip.
- Allow the strip to develop for 5 minutes before making final assay interpretations.
- 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 hay, dust, fluids, or disposables.

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 retested using another strip.

If the extract is from a sample containing approximately 5% (w/w) or more Roundup Ready alfalfa hay, a second pink line (Test Line) will develop on the membrane strip between the Control Line and the protective tape. **Any pink line in the Test Line area, no matter how light, 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 meant to be a qualitative test.
- The strips will detect Roundup Ready protein in sample extracts prepared following the specified extraction procedure at composite hay levels of approximately 5% (w/w) Roundup Ready alfalfa or more and is dependent upon the expression level of the Roundup Ready alfalfa plant.
- An example of a 5% (w/w) sample would be a mixed composite sample containing 5 grams of Roundup Ready alfalfa hay and 95 grams of conventional alfalfa hay.
- This product is currently not applicable for use in any other crop.
- 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.
- A negative result with this kit does not mean that the sampled tissue has not been otherwise genetically modified.
- It is not safe to conclude that a sample is negative before a full 5 minutes have elapsed.
- 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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