QuickStix™ Kit for Roundup Ready®
Canola Bulk Grain

Catalog Number AS 017 BG

Highlights:
- *Results in 5 minutes or less*
- *Available as 100-strip kits, or in bulk packaging*

Contents of Kit:
- *100 QuickStix Strips packed in two moisture-resistant canisters*
- *100 fixed volume transfer pipettes (250 microliters)*
- *100 reaction vials*

Items Not Provided:
- *Oster® Sunbeam blender, 4000 series or equivalent*
- *Ice blade with rubber gasket*
- *½ pint (8 oz.) Mini blender jars*
- *Threaded bottom cap*
- *Protective cover for blender jar while grinding*
- *Tap water*

Intended Use

The EnviroLogix QuickStix Kit for Roundup Ready Canola Bulk Grain is designed to extract and detect the presence of the Roundup Ready protein at the levels typically expressed in genetically modified canola. The sensitivity of this QuickStix Kit is 0.1% (i.e. one Roundup Ready seed in 1000 conventional canola seeds). For detection of Roundup Ready in canola plant tissues and individual seeds, please use QuickStix Cat# AS 017 LS.

How the Test Works

In order to detect the Roundup Ready protein with this QuickStix Kit, the sample must first be crushed and extracted with tap water to solubilize the protein.

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 reaction vial. 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

Step 1: Determine Number and Size of Sub-samples

- Collect a composite sample according to USDA/ GIPSA instructions found in the following reference documents:
  - **http://www.gipsa.usda.gov/fgis/handbook/gihbk1_inspec.aspx** - USDA Grain Inspection Handbook, Book 1, Grain Sampling.
  - **http://www.gipsa.usda.gov/fgis/biotech/sample2.htm** - Guidance document entitled Sampling for the Detection of Biotech Grains.
  - **http://www.gipsa.usda.gov/fgis/biotech/sample1.htm** - Practical Application of Sampling for the Detection of Biotech Grains.
  - **www.gipsa.usda.gov/fgis/biotech/samplingplan1.xls -** This website provides a simple to use Sample Planner (29K Excel Spreadsheet). The planner allows you to enter different assumptions in terms of sample size, number of samples, acceptable quality level and to determine the probability of accepting lots with given concentration levels. It also plots the probabilities in graph form for easy interpretation. Specific data can be saved for documentation and future analyses.
- The following is a helpful reference for use in designing a sampling plan: Remund, K.M., Dixon, D.A., Wright D.L., Holden, L.R. "Statistical considerations in seed purity testing for transgenic traits", Seed Science Research, June 2001, Vol. 11 No.2, pp. 101-119.
- To select the appropriate sample size, first determine the purity standard and the degree of confidence required. Confidence level means the statistical probability that the % of Roundup Ready in the lot is below the selected purity standard. Table 1 provides a guideline for determining the number of sub-samples necessary to provide effective screening for different GM concentrations at the 95% and 99% confidence levels.

To calculate the average seed weight:

- *Count & weigh 100 seeds*
- *Divide total weight by 100*

Weigh sample into blender jar, grind

Add calculated volume of water

Buffer Volume Formula
Grams canola seed x 5
= mL of tap water to add

For example: a 332-seed sample with an average weight of 0.0033g:
(332 x 0.0033 = 1.06 g x 5 = 5.3 mL tap water)

Shake to mix, allow to settle briefly

Table 1 – Canola - Number of seeds required

Testing 1 sub-sample with that sub-sample being negative (-)

Confidence Level (%)	Roundup Ready Screening Level		
	5%	3%	0.9%
95%	59	99	332
99%	90	152	510

Testing 2 sub-samples with 1 or both being negative (-/-) or (-/+)

	5%	3%	0.9%
95%	72	120	407
99%	104	174	586

Testing 3 sub-samples with 2 or 3 sub-samples being negative (-/-/-) or (-/-/+)

	5%	3%	0.9%
95%	39	66	221
99%	55	93	315

Note: Screening at the 0.1% Roundup Ready concentration level, with 95% confidence, requires 3 sub-samples of 1000 seeds with all sub-samples being negative (-/-/-).

For other sampling scenarios or different screening or confidence levels, refer to the USDA/GIPSA spreadsheet described under Step 1 above, or call EnviroLogix Technical Support for assistance.

Step 2: Determine Sub-sample Weight

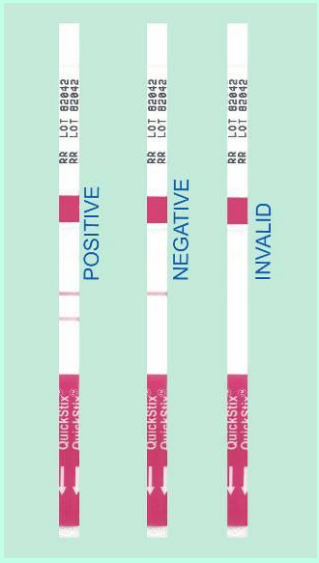
- Determine average weight of individual grain to be tested (see formula, above left).
- Calculate the sub-sample weight (g) needed for testing, (number of seeds X average seed weight).

Step 3: Prepare the Sample

- Weigh sample into the blender jar.
- Put rubber gasket then blade atop the jar.
- Attach black blender base to jar and hand tighten.
- Invert and put the blender jar on the blender motor, turn blender on high for 30 seconds.
- Remove the blender jar, invert and tap. Remove the base and blade assembly. Visually inspect to ensure that every seed has been broken. Add the volume of tap water calculated at left. For sample sizes of fewer than 200 seeds, use a minimum volume of 3.0 mL water to extract.
- Put white lid on the blender jar.
- Shake the sample for 30 seconds.
- Let sample settle (approximately 30 seconds). Dispense 250 µL extract into reaction vial using disposable fixed volume pipette provided with the kit; avoid particulates.

*Pipette sample into via*l

Add QuickStix and read the results



Any clearly discernable pink Test Line is considered positive

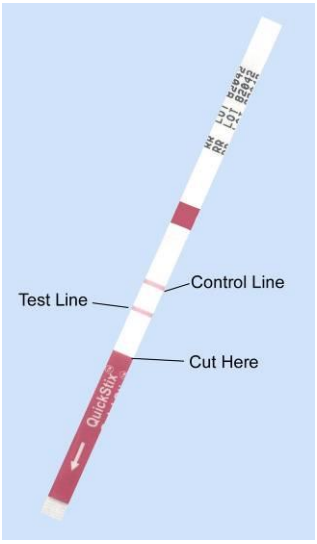
- To prevent cross-contamination, use a new transfer pipette and reaction vial for each sample.

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 reaction vial. The sample will travel up the strip. Reaction vials will stand on their own or may be inserted into the cardboard racks provided.
- 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.

NOTE: Use extreme caution to prevent sample-to-sample cross-contamination with grain, 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 re-tested using another strip.

If the extract is from a sample containing at least 0.1% Roundup Ready-modified canola, 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 the presence of Roundup Ready protein.*

If the extract is from a negative sample, the strip will only show the control line.

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.

Precautions and Notes

- This kit is designed to screen for presence or absence only, and is not meant to be quantitative.
- As with all tests, it is recommended that results be confirmed results by an alternate method if 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 diagnostic tool 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 strong positive result may safely be interpreted in fewer than 5 minutes after sample addition. It is not safe to interpret weak positive or negative results prior to 5 minutes.
- DO NOT leave in direct sunlight or in vehicle. Protect all components from hot or cold extremes of temperature when not in use.

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