ENVIROLOGIX™

# QuickStix™ Kit for Roundup Ready® Corn Bulk Grain

*Catalog Number AS 010 BG*

**Please Note**: Vial size and volume of extract has changed – fill vial to the ridge:

***Highlights:***

- *Use with Common Extraction™ method*
- *Results in 5 minutes or less*
- *Available as 100-strip kits, in bulk packaging, or in QuickCombs™*

***Contents of Kit:***

- *100 QuickStix Strips packed in two moisture-resistant canisters*
- *100 transfer pipettes*
- *100 reaction vials*

***Items Not Provided:***

- *Waring blender, model 31BL91 or equivalent*
- *Glass jar adapter (Eberbach #E8495)*
- *Glass Mason jars*
- *Graduated cylinder*
- *Tap water*
- *Protective cover for blender jar while grinding*

## Intended Use

The QuickStix Kit for Roundup Ready Corn Bulk Grain is designed to extract and detect the presence of CP4 EPSPS protein at the levels typically expressed in Roundup Ready corn. The sensitivity of these QuickStix Strips is 0.5% Event NK603 corn (i.e. one Event NK603 Roundup Ready corn kernel in 200 conventional corn kernels). This kit will not detect GA21 Roundup Ready corn.

## How the Test Works

In order to detect the CP4 EPSPS protein expressed by Roundup Ready corn, the sample must first be extracted 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." Please avoid bending the strips.

## Sample Preparation

### Step 1: Determine Number and Size of Sub-samples

1. Collect a composite sample according to USDA/GIPSA instructions found in the following reference documents:
   - **http://www.archive.gipsa.usda.gov/reference-library/handbooks/grain-insp/grbook1/bk1.pdf** - USDA Grain Inspection Handbook, Book 1, Grain Sampling.
   - **http://www.archive.gipsa.usda.gov/biotech/sample2.htm** - Guidance document entitled Sampling for the Detection of Biotech Grains.
   - **http://www.archive.gipsa.usda.gov/biotech/sample1.htm** - Practical Application of Sampling for the Detection of Biotech Grains.
   - **http://www.archive.gipsa.usda.gov/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.
2. 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.
3. To select the appropriate sample size, determine the purity standard and the degree of confidence required. Confidence level means the statistical probability that the true Roundup Ready level in the seed lot is below the selected purity standard. Table 1 provides a guideline for determining the number of kernels in

***Corn Common Extraction***
*Grams of Corn x 1.5=mL of water*
*For example:*
*(100 x 0.25)=25g x 1.5= 38mL water*

*Shake, wetting entire sample*

*Avoid pulling up particles when drawing sample*

*Fill vial to ridge with extract*

each sub-sample that are necessary to provide effective screening for different GM concentrations at the 95% and 99% confidence levels.

**Table 1 – Corn** Number of 200 kernel sub-samples required

| Confidence Level (%) | Roundup Ready Screening Level Event NK603 Corn | | | |
|---|---|---|---|---|
| | 5% | 1% | 0.5% | 0.25% |
| 95% | 1 | 2 | 3 | 6 |
| 99% | 2 | 3 | 5 | 9 |

Note: Screening at the 0.5% Event NK603 Roundup Ready concentration level, with 95% confidence, would require testing 3 sub-samples of 200 kernels with all sub-samples negative.

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

### Step 2: Determine Sub-sample Weight, Jar Size and Grind Times

1. Determine average weight of individual grain to be tested (weigh 100 seeds, divide by 100).
2. Calculate the weight of the number of grains to be tested (Number of grains X Average Weight/Grain). Table 2 lists the guidelines for jar size and grinding time according to sample weight.

**Table 2**

| Commodity | Sample Weight (g) | Jar Size (oz.) | Grind Time (sec.) |
|---|---|---|---|
| Corn | 10-25 | 4 | 30 |
| | 25-65 | 8 | 30 |

3. Choose an appropriate jar size for your sample based upon Table 2 above.

### Step 3: Prepare the Sample

1. Weigh sample into the appropriate size glass Mason jar.
2. Put protective cover over the jar attached to the blender.
3. Grind sample with a Waring blender (or equivalent) and jar adapter on high speed for specified grinding time or until all whole grains are broken.
4. Add the volume of tap water calculated by the formula at left.
5. Cap and shake jar vigorously until the entire sample is wet (20-30 seconds, depending on the number of grains). Sample will begin to settle immediately and liquid can be drawn off at that time.
6. Draw up liquid portion from above the settled sample and dispense extract into reaction vial until it is filled (this will take 2-3 transfers). Avoid pulling up particles. Allow extract to settle in the reaction vial for 30 seconds before adding a test strip.
7. To prevent cross-contamination thoroughly clean blender parts and jars of dust and residue prior to preparation of a second sample. Use a new transfer pipette and reaction vial for each sample.

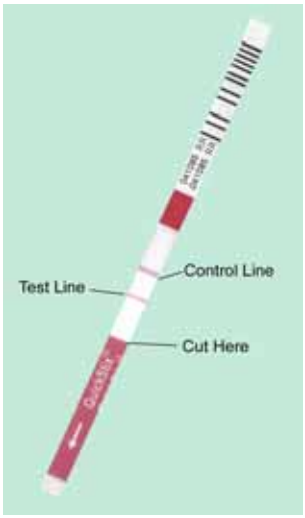
# How to Run the QuickStix Strip Test

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.
2. 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.

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 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 0.5% Event NK603 Roundup Ready corn (1 in 200), 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 show the control line. The result can not be taken to indicate that the sample is free of GA21 corn.


*Any clearly discernable pink Test Line is considered positive*

# 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.
- This product is currently not applicable for use in any other crop or in leaf or individual seed testing.
- 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 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.
- Warning: a strong positive result may safely be interpreted in as little as 2 minutes after sample addition. It is not safe, however, to interpret negative results prior to 5 minutes.
- Protect all components from hot or cold extremes of temperature when not in use.

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