

Catalog Number AS 015 BG

Highlights:

- 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

This EnviroLogix QuickStix™ Kit for YieldGard® Rootworm Bulk Grain is designed to extract and detect Cry3Bb protein at the levels typically expressed in YieldGard Rootworm corn. The sensitivity of these QuickStix strips is 0.5% (i.e. 1 kernel in a pool of 200).

How the Test Works

In order to detect the Cry3Bb protein expressed by YieldGard Rootworm corn, the sample must first be extracted in 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”. 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 % of YieldGard Rootworm corn 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.



Sample sizes

Calculate Water Volume for Corn Extraction

Sub-sample weight (g) x 1.5 = volume of water (mL)



Avoid pulling up particles when drawing sample

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

Confidence Level	Screening Level % YieldGard Rootworm Corn			
	5%	1%	0.5%	0.25%
95%	1	2	3	6
99%	2	3	5	9

NOTE: Screening corn at a 0.5% YieldGard Rootworm 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, Grind Times and Water Volume Needed for Sample Preparation

1. Determine the **average weight** of the grain from the lot to be tested. Count and weigh 100 kernels/seeds, then divide by 100.
2. Calculate the sub-sample weight (g) needed for testing, (200 seeds X **average seed weight**).
3. Choose an appropriate jar size for your sample based upon Table 2.
4. Calculate water volume needed for sample preparation. The water volume to sample weight is a ratio of **1.5 to 1**.

*Example Calculation using a 200 kernel sub-sample with an average kernel weight of 0.3g.
0.3g x 200 = 60 g x 1.5ml = 90 ml water for extraction*

Table 2

Commodity	Sub-sample Weight (g)	Jar Size (oz.)	Grind Time (sec.)
Corn	10-25	4	30
	25-65	8	30
	65-250	32	45

Step 3: Prepare the Sample

1. Weigh sample into the appropriate size glass Mason jar and attach jar adapter with blade.
2. Place unit on the Waring blender (or equivalent) and cover with protective cover.
3. Grind sample on high speed for specified grinding time or until all whole kernels are broken.
4. Add the volume of tap water calculated by the formula (above 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 enough liquid portions from above the settled sample to fill the long narrow tip of the transfer pipette and up to the line at the top of the flared portion of the pipette bulb (see figure at left). Avoid pulling up particles. Dispense extract into reaction vial.
7. To prevent cross-contamination, thoroughly clean blender parts and jars to remove dust and residue prior to preparation of each sample. Use a new transfer pipette and reaction vial for each sample.



How to Run the QuickStix Strip Test

1. Remove the QuickStix Strip from the canister and re-seal the canister. Insert the strip into the sample extract with the arrow tape pointing into the reaction vial.
2. After inserting the strip into the reaction vial, liquid will travel up the membrane strip toward the absorbent pad at the top of the strip. Soon after complete wetting of the membrane, a line will appear on the membrane approximately 1/4 inch below the top absorbent pad. This is the Control Line which should develop within 2-5 minutes. A positive result can be interpreted as soon as a Test Line develops; generally within 2-3 minutes. Allow the strip to develop for the full 5 minutes before concluding that the sample has tested negative.
3. To retain the strip, cut off the bottom section of the stick covered by the arrow tape.

NOTE: Reaction vials will stand alone or may be inserted into the cardboard rack provided in the shipment.

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% YieldGard Rootworm corn (1 kernel 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 Cry3Bb protein expression.*

If the extract is from a sample containing less than 0.5% YieldGard Rootworm corn, the strip will only develop a control line.



Any clearly discernable pink Test Line is positive

Kit Storage

This QuickStix kit should be stored at room temperature, or refrigerated for longer shelf life. Please 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.

Important: Do not open the desiccated canister until you are ready to use the strips. Allow refrigerated canisters to come to room temperature before opening to prevent condensation. Immediately re-seal unused QuickStix strips in the canister.

Cross-reactivity

The following materials have been tested with this kit and have been found to cause no false positive results:

- Proteins: Cry1Ab, Cry1Ac, Cry1C, Cry1F, Cry2Aa, Cry2Ab, CP4 EPSPS, Cry9C, PAT/*pat*, PAT/*bar*, and cyanamide hydratase at concentrations at least 4 times higher than reported in commercial crops.



- Other GMO Event Corns: YieldGard® Corn Borer (Bt11, MON810), Knockout®/NatureGard® (Bt176), LibertyLink® (T25), Starlink®, Roundup Ready (Event GA21), Roundup Ready (Event 603), and Herculex™ I corn bulk grain.
- Other Crops: Bulk grain from conventional varieties of corn (yellow or white) and sorghum at 100% of sample; barley, canola, rice, and sugarbeets at 50% of sample; soybean and wheat at 25% of sample; and cotton at 10% of sample.

Precautions and Limitations

- 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.
- As with all tests, it is recommended that results be confirmed 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.
- Warning: a strong positive result may be safely 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. Do not leave in direct sunlight or in vehicles.
- Use extreme caution to prevent sample-to-sample cross-contamination with fluids or disposables.



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