ENVIROLOGIX™

QuickStix™ Kit for Cry1F Bulk Grain

Catalog Number AS 016 BG

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 Cry1F is designed to extract and detect the presence of the Bt endotoxin Cry1F in Herculex™ I and HERCULEX XTRA Insect Protection traits. The sensitivity of this QuickStix Kit is 0.5% based on tests conducted with Herculex I corn (i.e. one kernel out of 200). For Cry1F detection in corn leaf tissues and individual seeds, please use QuickStix Cat# AS 016 LS.

How the Test Works

Corn crops that have been genetically modified with a *cry1F* gene express Cry1F protein in their tissue. To detect the protein, samples must first be ground and extracted in tap water to solubilize the endotoxins.

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:
   - **www.gipsa.usda.gov/publications/fgis/handbooks/gihbk1_insphb.html** - USDA Grain Inspection Handbook, Book 1, Grain Sampling.
   - **www.gipsa.usda.gov/fgis/biotech/sample2.htm** - Guidance document entitled Sampling for the Detection of Biotech Grains.
   - **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 -** 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 Cry1F level in the seed 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

Corn
Grams of Corn x 1.5= mL of water


Shake, wetting entire sample


Avoid pulling up particles when drawing sample

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

Confidence Level (%)	Cry1F Screening Level			
	5%	1%	0.5%	0.25%
95%	1	2	3	6
99%	2	3	5	9

Note: Screening at the 0.5% Cry1F 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
	65-250	32	45

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

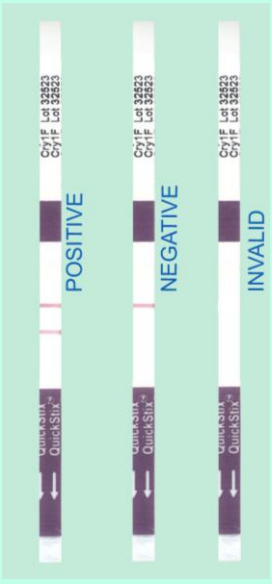
Step 3: Prepare the Sample

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. *For example: If testing 100 kernels with an average weight of 0.25g: (100 x 0.25)=25g x 1.5=38mL water.*
5. Cap the jar and shake vigorously for at least 30 seconds, or longer if needed, to thoroughly wet all of the corn in the sample. 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 to remove 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

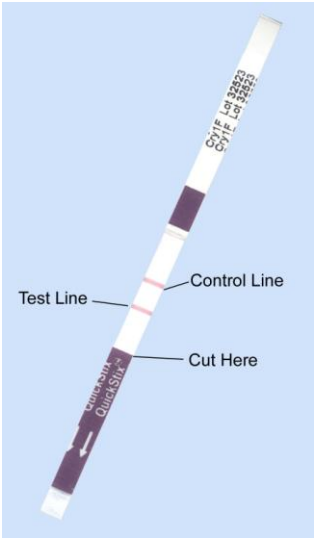
1. Allow refrigerated canisters to come to room temperature before opening. Remove the QuickStix Strips to be used. Avoid bending the strips. Reseal the canister immediately.
2. Place the strip into the reaction vial. The sample will travel up the strip. Reaction vials will stand on their own or may be inserted into the cardboard racks provided.

Fill vial to ridge with extract



Any clearly discernable pink Test Line is considered positive

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.



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% Cry1F-modified corn, 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 the presence of Cry1F 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.
- 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 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.
- Warning: a strong positive result may safely be interpreted in as little as 2 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.
- **Use extreme caution to prevent sample-to-sample cross-contamination with grain, fluids, or disposables.**

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