

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 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



Weigh the sample into a glass Mason jar

Catalog Number AS 037 BG

Intended Use

The EnviroLogix QuickStix Kit for mCry3A is designed to extract and detect the presence of the modified Cry3A protein at the levels typically expressed in genetically modified corn grain. In a ground corn sample the kit will detect concentrations of 0.75% or greater of mCry3A corn. For mCry3A detection in corn leaf tissue, please use QuickStix Cat# AS 037 LT.

How the Test Works

In order to detect the mCry3A proteins with this QuickStix Kit, the sample must first be ground and 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 which end of the strip to insert into the reaction vial. 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

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_inspbh.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 ensure accurate detection of mCry3A corn in bulk grain, two 300-kernel samples must be tested separately. A negative test result for both samples enables the tester to conclude with 95% confidence that mCry3A corn is present at less than 1% in the sampled population. This sampling recommendation is based on the mCry3A protein expression profile in harvested grain of Agrisure RW.



Grind

Corn Common Extraction

Grams of Corn x 1.5 = mL of water

For example:

$$(100 \times 0.25) = 25\text{g} \times 1.5 = 38\text{mL water}$$



Shake, wetting entire sample



Avoid pulling up particles when drawing sample



Fill vial to ridge with extract

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: Prepare the Sample

1. Determine average weight of the grain to be tested (weigh 100 kernels, divide by 100), then multiply the average weight by the desired sample size (for example average weight of 0.3g x 300 kernels would require a 90g sample).
2. Weigh sample into a 32-ounce glass Mason jar and attach jar adapter with blade.
3. Place assembly on the Waring blender (or equivalent). Shield with protective cover to prevent injury in the event of jar breakage.
4. Grind sample at high speed for at least 45 seconds, or until all whole grains are finely ground.

NOTE: Alternatively, a Bunn grinder or similar milling device may be used as long as it grinds the kernels to an equivalent fine grind.

5. Add the volume of tap water calculated by the formula at left. For example: if testing 300 kernels with an average weight/kernel of 0.3 g: $(300 \times 0.3) = 90 \text{ g} \times 1.5 = 135 \text{ mL water}$.
6. Cap the jar and shake vigorously for at least 90 seconds, or longer if needed, to thoroughly wet all of the corn in the sample. Allow sample to settle briefly.
7. 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.
8. 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.
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 promptly at 5 minutes and discard the bottom section of the strip covered by the arrow tape.

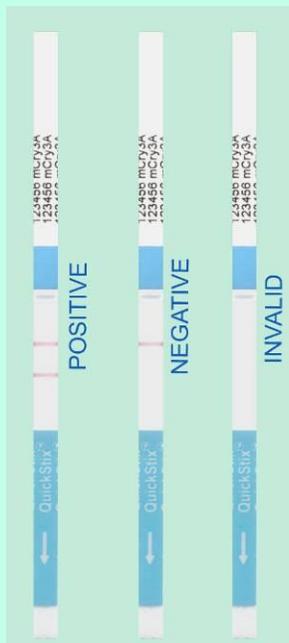
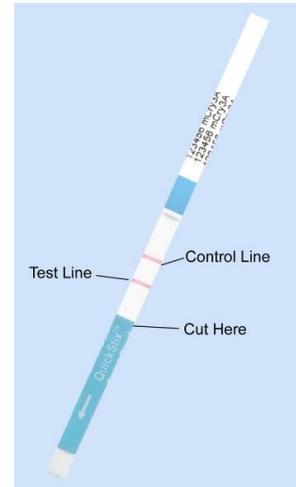


Interpreting the Results

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 0.75% mCry3A-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 modified Cry3A protein.*

If the extract is from a negative sample, the strip will only show the Control Line.



Any clearly discernable pink Test Line is considered positive



Kit Storage

QuickStix Strips 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 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 as little as 2 minutes after sample addition. It is not safe, however, to conclude that a sample is negative before a full 5 minutes has elapsed. A weakly positive sample may require the full 5 minutes for a distinct Test Line to appear.
- Protect all components from hot or cold extremes of temperature when not in use. Do not leave in direct sunlight or in vehicle.
- Use extreme caution to prevent sample-to-sample cross-contamination with grain, fluids, or disposables.



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