

Catalog Number AS 008 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 EnviroLogix QuickStix Kit for Cry9C is designed to extract and detect the presence of the Cry9C protein in StarLink™ and other corn varieties in bulk corn grain. In a ground corn sample, the kit will detect concentrations of 0.125% or greater of Cry9C corn. For use with corn meal, flour and grits, call EnviroLogix for the appropriate application guide. For Cry9C detection in corn plant tissues and in individual seeds, please use QuickStix Cat# AS 008 LS.

NOTE: A negative result with this test on corn seed or grain extracts does not necessarily rule out the presence of genetically modified material in the sample.

How the Test Works

Corn crops that have been genetically modified with Cry9C gene express a specific type of Bt protein in their tissues. To detect the Cry9C protein with the EnviroLogix QuickStix Kit for Cry9C, grain samples must be extracted and the Cry9C protein solubilized in water.

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.

Selecting a Sampling Protocol

The sampling scheme can dramatically affect the probabilities and level of confidence in detecting any StarLink corn that may be commingled in the sample. The chart that follows shows the probability of detecting a given concentration of StarLink corn in the sample, and how the probability of detection is increased with multiple samples. For example, if testing one sub-sample of 800 kernels, a concentration of 0.37% StarLink corn can be detected with 95% confidence. Testing two sub-samples of 800 kernels each will provide a 95% confidence of detecting concentrations of 0.19% or greater. Testing three sub-samples of 800 kernels each will provide detection at the 0.12% level with 95% confidence. Stated another way, your confidence in detecting a specific concentration increases with the number of sub-samples. For example, at concentrations of 0.2% or greater, testing one 800-kernel sub-sample would provide ~80% confidence in detection, testing two 800-kernel sub-samples would provide ~96% confidence in detection, and testing three 800-kernel sub-samples would provide detection at the 99% confidence level.

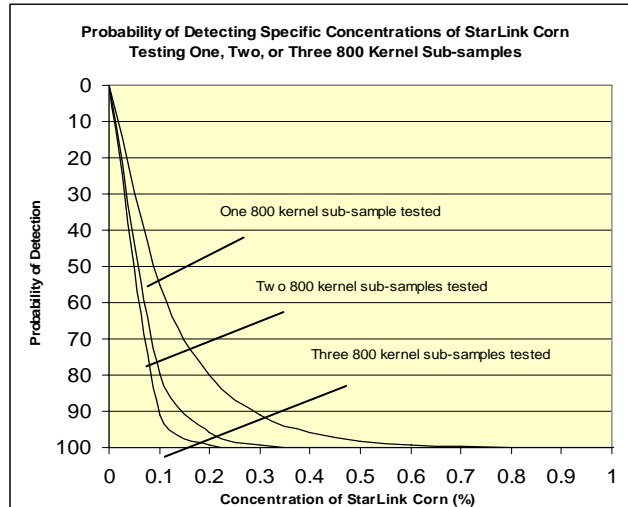
For further information on USDA/GIPSA guidelines for obtaining representative samples and assessing detection probabilities for biotech grain, see the following websites:

<http://archive.gipsa.usda.gov/reference-library/handbooks/grain-insp/grbook1/bk1.pdf> -- USDA Grain Inspection Handbook, Book 1, Grain Sampling. This document provides a comprehensive overview of recommended sampling guidelines for static lots and grain streams. It reviews the various types of equipment and strategies that can be used to obtain a representative grain sample from different types of containers.

<http://archive.gipsa.usda.gov/biotech/sample2.htm> -- Guidance document entitled *Sampling for the Detection of Biotech Grains*, which provides important statistical sampling considerations when testing for the presence of biotech grains. It covers the basis for making probability determinations in accepting lots based upon different assumptions with respect to sample size, number of samples, sample preparation, etc.

<http://archive.gipsa.usda.gov/biotech/sample1.htm> -- *Practical Application of Sampling for the Detection of Biotech Grains*. This one-page application guide provides a table that gives sample sizes for selected lot concentrations and probability of rejecting the specified concentrations. It also provides a formula for making the calculation for other combinations.

<http://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.



The chart below provides an indication of the maximum concentration of StarLink corn expected to be in the sample relative to the number of sub-samples tested, assuming all samples test negative. The maximum concentrations (percent) of StarLink expected in the sample are provided for both the 95% and 99% confidence levels. As can be seen, increasing the number of sub-samples tested increases the confidence of detection at increasingly lower concentrations in the sample. Testing six sub-samples of 800 kernels each, with all results being negative, would indicate with 99% confidence that there is no more than 0.10% StarLink corn in the sample.

Maximum Percentage of Cry9C in Sample (%)

Number of 800-Kernel Sub-samples	At 95% Confidence Level	At 99% Confidence Level
1	0.37	0.57
2	0.19	0.29
3	0.12	0.19
4	0.09	0.14
5	0.07	0.12
6	0.06	0.10

Sample Preparation

NOTE: Thorough mixing of the bulk grain sample and determination of an appropriate sampling plan are critical to the results of this testing, and are the responsibility of the user of this test kit. The USDA/GIPSA has prepared several guidance documents to address the issues involved in obtaining representative grain samples from static lots - such as trucks, barges, and railcars - and for taking samples from grain streams. Sampling plans should be chosen that best meet the needs of both the buyer and seller in terms of acceptable risks. As indicated in the previous section *Selecting a Sampling Protocol*, increasing the number of kernels in the sample and taking multiple samples will increase the likelihood of obtaining representative samples, and maximize the probability of detecting any contamination in the grain lot. For further information on USDA/GIPSA guidelines for obtaining representative samples and assessing detection probabilities for biotech grain, see the websites listed at left.

The EnviroLogix Cry9C QuickStix Kit will detect one positive kernel in an 800-kernel sample (0.125% concentration of StarLink corn). To maximize the likelihood of detecting any Cry9C (StarLink) corn commingled in the lot, it is recommended that multiple 800-kernel sub-samples, as representative of the lot as possible, each be tested as follows:



Corn
Grams of Corn x 1.5 =
mL of water



Avoid pulling up particles
when drawing sample



1. Obtain a representative sample of the desired size. By determining the average weight per kernel (weigh 100 seeds, divide by 100), samples can be measured by total weight. For example, if the average weight per kernel is 0.3 grams, an 800-kernel sample can be obtained by weighing out 240 grams of corn.
2. Weigh sample into an appropriate sized glass Mason jar (32 oz. for 800-kernel samples).
3. Put a protective cover over the jar attached to the blender.
4. Grind sample with a Waring blender (or equivalent) and jar adapter on high speed until all whole kernels are broken.
5. Add distilled, deionized or tap water to the ground corn according to the following chart:

Avg. Seed Weight	Weight of 800 seeds	Water to add
0.2g	160g	240 mL
0.25g	200g	300 mL
0.3g	240g	360 mL
0.35g	280g	420 mL

6. 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.
7. Draw up enough liquid portion from above the settled sample to fill the long narrow tip of the transfer pipette up to the line at the top of the flared portion of the pipette bulb (see illustration). Avoid pulling up particles. Dispense extract into reaction vial.
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.

NOTE: For different sample sizes, the amount of water to use in extracting the sample should be 1.5 x the gram weight of the sample. When using the Common Extraction protocol to test for other traits in the same ground sample, the amount of water to use in extracting the sample should be 1.5x the gram weight of the 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.

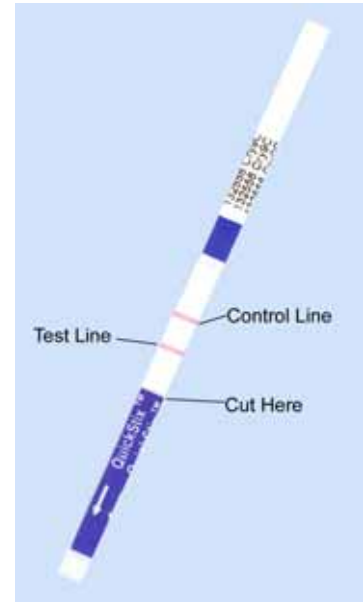
Interpreting the Results

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 sample extract contains at least 0.125% Cry9C-modified (StarLink) 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 Cry9C protein expression. (Based upon tests conducted with known positive and negative corn provided by Aventis Crop Science, the developer of StarLink corn.)*

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

In 5 minutes the test will detect 1 positive kernel in an 800-kernel sample, with 799 negative kernels (0.125%).



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 Limitations

- This kit is designed for screening 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 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.
- A negative result with this kit does not mean that the sampled grain has not been otherwise genetically modified.
- 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.



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