ENVIROLOGIX™

QuickStix™ Kit for QuickScan - Cry1F Corn Bulk Grain

Catalog Number AQ 016 BG

Highlights:

- *Use with Common Extraction™ method*
- *Quantitative results in just over 5 minutes*
- *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 cups*

Items Not Provided:

- *Waring blender, model 31BL91 or equivalent, with glass jar adapter (Eberbach #E8495) and glass Mason jars*
  ~~OR~~
- *Bunn grinder or equivalent*
- *Graduated cylinder*
- *Tap water*
- *Protective cover for blender jar while grinding*
- *QuickScan System*

Intended Use

The QuickStix™ Kit for QuickScan – Cry1F detects and quantifies the Bt endotoxin Cry1F in Herculex™ I and HERCULEX XTRA Insect Protection traits. The Limit of Detection for these QuickStix Strips is 0.5% based on tests conducted with Herculex I corn (i.e. one kernel out of 200).

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 cup. 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 interpret the reactions as described under "Interpreting the Results." Results are scanned and interpreted quantitatively with the EnviroLogix QuickScan System. Please avoid bending the strips.

Sample Preparation

1. Collect a composite sample according to USDA/ GIPSA instructions found in the reference documents listed in the margin on Page 2.
2. Determine the **average weight** of the grain from the lot to be tested. Count and weigh 100 kernels/seeds, then divide by 100.
3. Calculate the sub-sample weight (g) needed for testing, (number of seeds X **average seed weight)**.
4. Choose appropriate container based on sub-sample weight. If using Waring blender, container must allow enough free space above the sample for blades to operate.
5. Calculate water volume needed for sample preparation. The Common Extraction Method calls for a water volume to sample weight ratio of **1.5 to 1.**

Example Calculation using a 400 kernel sub-sample with an average kernel weight of 0.3g.
0.3g x 400 =120g x 1.5mL =180 mL water for extraction

Grind Sub-Sample:

Bunn grinder or equivalent:

6. Weigh out subsample based on the average weight per seed calculation.
7. Grind subsample (using Auto-Drip setting if using a Bunn grinder) with grinder until all whole grains are broken. The sample should be the consistency of coffee grounds.

Waring blender or equivalent:

6. Weigh sample into the appropriate size glass Mason jar and attach jar adapter with blade.
7. Place assembly on the Waring blender (or equivalent). Shield with protective cover to prevent injury in the event of jar breakage. Grind sample at high speed for 15-45 seconds, or until all whole grains

USDA References:

- **http://archive.gipsa.usda.gov/reference-library/handbooks/grain-insp/grbook1/bk1.pdf** - USDA Grain Inspection Handbook, Book 1, Grain Sampling.
- **http://archive.gipsa.usda.gov/biotech/sample2.htm** - Guidance document entitled Sampling for the Detection of Biotech Grains.
- **http://archive.gipsa.usda.gov/biotech/sample1.htm** - Practical Application of Sampling for the Detection of Biotech Grains.
- **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.

Corn Common Extraction™

Grams of Corn x 1.5=mL of water

For example, 400 kernels with an average seed weight of 0.3 g:

(400 x 0.3)=120 g of corn
120 g x 1.5=180 mL water

Transfer 20 mL extract to cup:

Either pour…

…or pipette to the 20 mL mark

***Bunn grinder or equivalent** (cont.):*

8. Place subsample into an appropriately sized jar or zip-type plastic bag and add the volume of tap water calculated using the Corn Common Extraction formula (left).
9. Cap the jar or "zip" plastic bag and shake vigorously for 30 seconds, then allow sample to settle for another 30 seconds.

***Waring blender or equivalent** (cont.):*

are broken. Optimum grind time may vary based on sample size and condition of equipment. The sample should be the consistency of coffee grounds.

8. Add the volume of tap water calculated using the Corn Common Extraction formula (left).
9. Cap the jar and shake vigorously for 30 seconds, then allow the sample to settle for another 30 seconds.

10. Transfer 20 mL of the liquid portion from above the settled sample into the sample cup. Pour extract into cup to the 20 mL line, or use a fresh pipette from the kit to transfer extract until the 20 mL line is reached (when testing smaller subsamples). **Important:** Avoid transferring particles as much as possible, and after transfer, **allow the liquid in the sample cup to settle for 30 seconds** so that any particles will settle at the bottom of the cup.
11. 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 cup 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 cup provided, being sure to insert the end indicated by the arrows on the protective tape. The sample will travel up the strip.
3. Allow the strip to develop for 5 minutes before making final assay interpretations. Positive sample results may become obvious much more quickly.
4. Immediately cut off and discard the bottom section of the strip covered by the arrow tape and place in the QuickScan Reader. Strips must be read while still wet.

NOTE: Use extreme caution to prevent sample-to-sample cross-contamination with grain, fluids, or disposables.

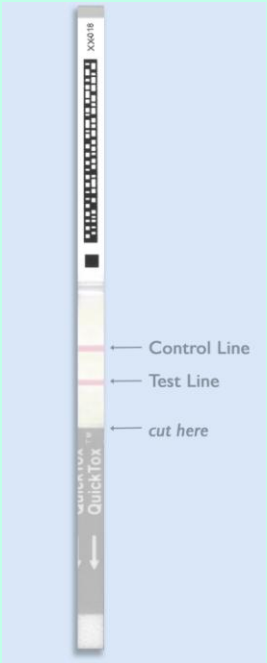
Interpreting the Results

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.

Results are scanned and interpreted quantitatively with the QuickScan System. Place QuickStix Strip into the carrier, slide in, and press "Read Test" on the screen. QuickScan will return a result as "% GMO" or "<LOQ" (less than the Limit of Quantification). Please consult the QuickScan User Manual for details.

(outlined to demonstrate cup size and markings)

After 30 seconds, add QuickStix to cup



Strip must develop a Control Line to be valid – cut where indicated and read in QuickScan System

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 to be quantitative using the QuickScan System.
- 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 kit 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.
- Protect all components from hot or cold extremes of temperature when not in use. Do not leave in direct sunlight or in a vehicle.

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