ENVIROLOGIX™

QuickStix™ Kit for QuickScan - Cry1F
Corn Bulk Grain

Catalog Number AQ 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 cups*

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*
- *QuickScan System (optional, for quantitative results)*

For sampling scenarios at different screening or confidence levels, refer to the USDA/GIPSA Excel spreadsheet described below, or call EnviroLogix Technical Support for assistance.

Intended Use

The QuickStix™ Kit for QuickScan – 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).

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 view the reactions as described under "Interpreting the Results." Results may then be scanned and interpreted quantitatively with the EnviroLogix QuickScan System. 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 reference documents listed in the margin on Page 2.
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.

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 corn at a 0.5% Cry1F concentration level, with 95% confidence, would require testing 3 sub-samples of 200 kernels with all sub-samples negative.

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:
(100 x 0.25)=25g x 1.5= 38mL water

Transfer extract to cup, about 3-4 pipettefuls

Avoid pulling up particles when drawing sample

Step 2: Determine Sub-sample Weight, Jar Size and Grind Times

1. Determine the **average weight** of the individual grain to be tested (count and 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	Sub-sample Weight (g)	Jar Size (oz.)	Grind Time (sec.)
Corn	10-25 25-65	4 8	30 30

Step 3: Prepare the Sample

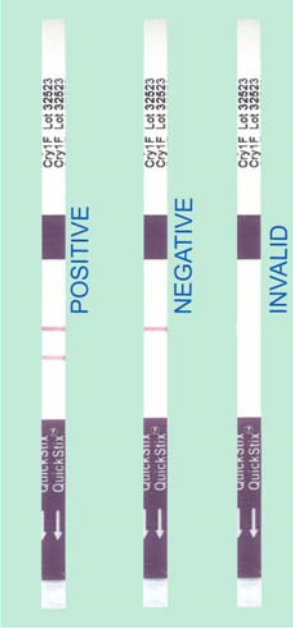
1. Weigh sample into the appropriate size glass Mason jar and attach jar adapter with blade.
2. Place unit on the Waring blender (or equivalent) and cover with protective cover.
3. Grind sample on high speed for specified grinding time or until the sample has the texture of coffee grounds.
4. Add the volume of tap water calculated by the Common Extraction formula at left. *For example: If testing 200 kernels with an average weight of 0.3g: (200 x 0.3)=60g x 1.5=90mL 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. If intending to read the test using QuickScan, be sure to shake for at least 30 seconds, and for accurate results, allow sample to settle for another 30 seconds.
6. Transfer 12 mL of the liquid portion from above the settled sample into the sample cup. The correct depth should be above the bottom of the arrows but below the top of the arrows printed on the combs. Avoid pulling up particles.
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 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. To retain the strip, or for use in the QuickScan System, cut off and discard the bottom section of the strip covered by the arrow tape. If reading the test using QuickScan, remember that strips must be read immediately after cutting, while still wet.

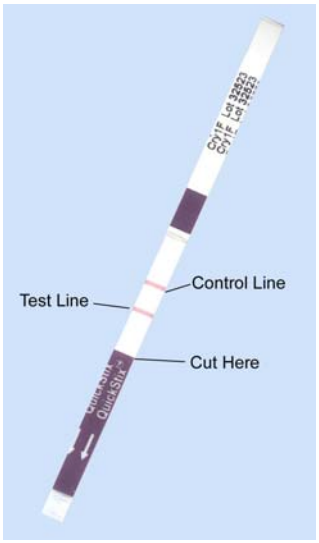
NOTE: Use extreme caution to prevent sample-to-sample cross-contamination with grain, fluids, or disposables.

Insert strip into cup—be sure liquid level is at or above the arrows' tips but below the top of the arrow



Any clearly discernable pink Test Line is positive

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 (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 Cry1F protein expression.*

If the extract is from a sample containing less than 0.5% Cry1F-modified corn, the strip will only develop a control line.

Results may then be scanned and interpreted quantitatively with the QuickScan System. Please consult the QuickScan User Manual for details.

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 read visually as a screen for presence or absence, and is also designed to be quantitative when used with 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.
- A strong positive result may safely be 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 a vehicle.

For Technical Support Contact Us At:

EnviroLogix
500 Riverside Industrial Parkway
Portland, ME 04103-1486
USA

Tel: (207) 797-0300
Toll Free: 866-408-4597
Fax: (207) 797-7533

e-mail:
info@envirologix.com

website:
www.envirologix.com

LIMITED WARRANTY

EnviroLogix Inc. ("EnviroLogix") warrants the products sold hereunder ("the Products") against defects in materials and workmanship when used in accordance with the applicable instructions for a period not to extend beyond a product's printed expiration date. If the Products do not conform to this Limited Warranty and the customer notifies EnviroLogix in writing of such defects during the warranty period, including an offer by the customer to return the Products to EnviroLogix for evaluation, EnviroLogix will repair or replace, at its option, any product or part thereof that proves defective in materials or workmanship within the warranty period.

ENVIROLOGIX MAKES NO OTHER WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO ANY IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The warranty provided herein and the data, specifications and descriptions of EnviroLogix products appearing in EnviroLogix published catalogues and product literature are EnviroLogix' sole representations concerning the Products and warranty. No other statements or representations, written or oral, by EnviroLogix' employees, agents or representatives, except written statements signed by a duly authorized officer of EnviroLogix Inc., are authorized; they should not be relied upon by the customer and are not a part of the contract of sale or of this warranty.

EnviroLogix does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by EnviroLogix, or against damages resulting from such non-EnviroLogix made products or components. EnviroLogix passes on to customer the warranty it received (if any) from the maker thereof of such non-EnviroLogix made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by EnviroLogix.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of EnviroLogix shall be to repair or replace the defective Products in the manner and for the period provided above. EnviroLogix shall not have any other obligation with respect to the Products or any part thereof, whether based on contract, tort, strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall EnviroLogix be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of EnviroLogix with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.