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 EnviroLogix QuickStix Kit for Cry1F is designed to extract and detect the presence of the Cry1F protein at the levels typically expressed in genetically modified corn grain. The sensitivity of this QuickStix Kit is 1.0% based on tests conducted with Herculex™ corn (i.e. one kernel out of 100). For Cry1F detection in corn and cotton plant tissues and individual seeds, please use QuickStix Cat# AS 016 LS.

How the Test Works

In order to detect the Cry1F 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 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:
   - **http://www.archive.gipsa.usda.gov/reference-library/handbooks/grain-insp/grbook1/bk1.pdf** - USDA Grain Inspection Handbook, Book 1, Grain Sampling.
   - **http://www.archive.gipsa.usda.gov/biotech/sample2.htm** - Guidance document entitled Sampling for the Detection of Biotech Grains.
   - **http://www.archive.gipsa.usda.gov/biotech/sample1.htm** - Practical Application of Sampling for the Detection of Biotech Grains.
   - **http://www.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.
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

Sample sizes

provides a guideline for determining the total number of kernels from sub-samples that are necessary to provide effective screening for different GM concentrations at the 95% and 99% confidence levels.

Table 1 – Corn - Number of kernels required

Confidence Level (%)	Cry1F Screening Level				
	5%	4%	3%	2%	1%
95%	59	74	99	149	299
99%	90	113	152	228	450

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.

Corn
Grams of Corn x 1.5= mL of water

Table 2

Commodity	Sample Weight (g)	Jar Size (oz.)	Grind Time (sec.)
Corn	10-25 25-65 65-250	4 8 32	30 30 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 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, left). Avoid pulling up particles. Dispense extract into reaction vial.
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.

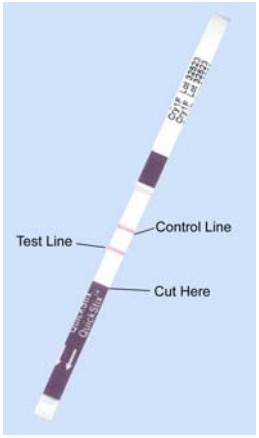
Avoid pulling up particles when drawing sample

How to Run the QuickStix Strip Test

1. Remove the QuickStix Strip from the canister and reseal the canister. Insert the strip into the sample extract with the arrow tape pointing into the reaction vial.
2. After inserting the strip into the reaction vial, liquid will travel up the membrane strip toward the absorbent pad at the top of the strip. Soon after complete wetting of the membrane, a line will appear on the membrane approximately 1/4 inch below the top absorbent pad. This is the Control Line which should develop within 2-5 minutes. A positive result can be interpreted as soon as a Test Line develops; generally within 2-3 minutes. Allow the strip to develop for the full 5 minutes before concluding that the sample has tested negative.
3. To retain the strip, cut off the bottom section of the strip covered by the arrow tape.

NOTE: Reaction vials will stand alone or may be inserted into the cardboard rack provided in the shipment.

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 1% 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.

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.

Cross-reactivity

The following materials have been tested with this kit using the protocols specified herein, and have resulted in no false positives:

- Other GMO Event Corns: YieldGard® Corn Borer (Bt11, MON810), Knockout®/NatureGard® (Bt176), LibertyLink® (T25), StarLink® (Cry9C), Roundup Ready® (Event GA21), Roundup Ready (Event 603), YieldGard Rootworm (Cry3Bb), and modified Cry3A corn bulk grain.
- Other Crops: Bulk grain from conventional varieties of corn, rice and sorghum at 100% of sample; barley and wheat at 50% of sample; soybean and sugarbeet at 25% of sample; and canola and cotton at 5% of sample.

Precautions and Limitations

- 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.**

For Technical Support Contact Us At:

Envilab GmbH
Cyriakusstraße 27

47839 Krefeld

Tel: (02151) 5671549

Fax: (02151) 5695602

e-mail:
info@envilab-gmbh.de

website:
www.envilab-gmbh.de

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.

Herculex is a trademark of Dow AgroSciences LLC
Roundup Ready and YieldGard are registered trademarks of Monsanto Technology, LLC
Knockout is a registered trademark of Novartis AG
NatureGard is a registered trademark of Mycogen Corporation
LibertyLink is a trademark of Bayer
Starlink is a trademark of Aventis Crop Science
EnviroLogix, the EnviroLogix logo, QuickComb, QuickStix and Common Extraction are trademarks of EnviroLogix Inc.