

Catalog Number AS 014 BG

Intended Use

The QuickStix Kit for LibertyLink is designed to extract and detect PAT/*pat* protein at levels typically expressed in T25 LibertyLink corn. The sensitivity of these strips is 0.5% (i.e. 1 kernel out of 200).

Please Note: Test now uses vial instead of cup -- fill vial to the ridge:



Highlights:

- 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



Sample sizes

How the Test Works

In order to detect the PAT/*pat* protein expressed by LibertyLink corn, the sample must first be 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.”

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 % of LibertyLink corn in the 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 (%)	LibertyLink Screening Level			
	5%	1%	0.5%	0.25%
95%	1	2	3	6
99%	2	3	5	9



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

Note: Screening at the 0.5% concentration level, with 95% confidence, would require testing 3 sub-samples of 200 kernels with all sub-samples negative.

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.

Table 2

Commodity	Sample Weight (g)	Jar Size (oz.)	Grind Time (sec.)
Corn	10-25	4	30
	25-65	8	30

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

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.



*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% T25 LibertyLink 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 PAT/pat protein expression.*

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

Note: This test **will** detect PAT/pat proteins such as those found in varieties other than LibertyLink, including but not limited to YieldGard (Bt11 and PAT/pat), Herculex RW (Cry34), and Herculex XTRA (Cry1F/Cry34) corn; and LibertyLink (PAT/pat) canola. However, because of the variation of PAT/pat expression levels in these different varieties, the detection level will differ (ie, it could take more than 1% inclusion of these varieties in a conventional lot to cause a positive reaction).

It **will not** detect PAT/bar proteins as expressed in StarLink corn and LibertyLink PAT/bar canola or cotton.



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 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.
- 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.
- Warning: a strong positive result may be safely 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 vehicles.
- Use extreme caution to prevent sample-to-sample cross-contamination with fluids or disposables.



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



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