QuickStix™ Kit for QuickScan LibertyLink® Bulk Soybeans

Highlights:

- *Results in 5 minutes or less*
- *Both bulk soybean kits utilize Common Extraction™:*
  - *Roundup Ready®*
  - *LibertyLink®*
- *Available as 100-strip kit, in bulk packaging, or in QuickCombs™*

Contents of Kit:

- *100 QuickStix Strips packed in two moisture-resistant canisters*
- *100 transfer pipettes*
- *100 three-ounce cups*

Items Not Provided:

- *Blender for sample prep:*
  1. *Oster® Sunbeam blender model #4094 (with 4 oz. polystyrene blender jar, ice crusher blade, gasket, and blender base)* ***~or~***
  2. *Waring blender model 31BL91 or equivalent (with glass Mason jars and jar adapter [Eberbach #E8495] along with protective cover* ***~or~***
  3. *BUNN coffee grinder (industrial style grinder set on AutoDrip setting)*
- *Graduated cylinder*
- *Tap water*
- *QuickScan System (optional, for quantitative results)*

Catalog Number AQ 014 BGB

Intended Use

This EnviroLogix QuickStix Kit for QuickScan - LibertyLink Bulk Soybeans is designed to extract and detect the presence of PAT/*pat* protein at the levels typically expressed in soybean event LL27. The sensitivity of these QuickStix Strips is 0.5% LibertyLink soybean (i.e. one soybean in 200).

How the Test Works

In order to detect the PAT/*pat* protein expressed by LibertyLink soybeans, the sample must first be extracted 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 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 following reference documents:
   - **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.
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 first determine the purity standard and the degree of confidence required. Confidence level means the statistical probability that the true *pat* soybean level in the sample is below the selected purity standard.
4. Table 1 provides a guideline for determining the number of 200 bean sub-

samples necessary to provide effective screening for different GM concentrations at a 95% confidence level.

Table 1 - Number of 200 soybean subsamples required for testing

Confidence Level (%)	LibertyLink PAT/*pat* (Event LL27) Soybean Screening Level	
	0.75 %	0.5 %
95%	2	3

Choose grinding method

Soybean Common Extraction
Grams of beans x 5 = mL water
For example: (100 x 0.15)=15g
15 g x 5 = 75 mL water

Extract in tap water

Transfer extract to cup, about 3 pipettefuls

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

1. Determine average weight of individual bean lot to be tested (weigh 100 beans, divide by 100).
2. Calculate the weight of the number of beans to sub-sampled and tested (number of beans X Average Weight/bean).
3. Choose an appropriate jar size and grind time based on the type of blender available for sub-sample preparation (see Table 2). Oster Sunbeam Blender with ice crusher blade is recommended over the Waring Blender for its bean grinding efficiency. (Note that bean grind time is longer and requires additional steps* when using a Waring Blender).

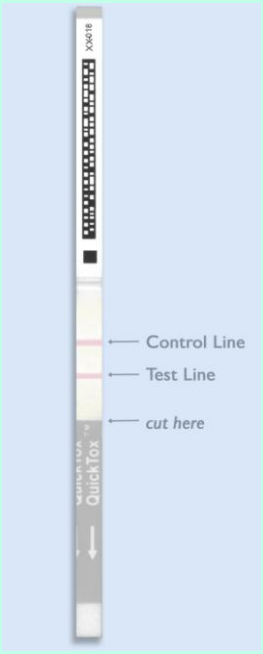
Table 2 - Soybeans

# of Beans (approximate)	Blender Type	Sub-sample weight (g)	Jar size (oz.)	Grind Time on High Speed
100-200	Oster Sunbeam	16-38	8	20 seconds
100	Waring	16-38	8	60 seconds (2 X 30 sec.*)
200-400	Waring	38-65	16	60 seconds (2 X 30 sec.*)

* For best results blend beans for ½ of total time, remove the jar and shake to redistribute larger particles, replace and resume grinding.

Prepare the sample

1. Weigh sample into the appropriate vessel.
2. Put protective cover over glass jars.
3. Grind sample on high speed until all whole beans are finely ground.
4. Add the volume of tap water calculated by the formula (above, left).
5. Cap and shake jar vigorously until the entire sample is wet (20-30 seconds, depending on the number of grains). Sample will begin to settle immediately and liquid can be drawn off at that time. Avoid pulling up particles with the transfer pipette.
6. Transfer 12 mL of the liquid extract from above the settled soybean solids into the 3 oz. sample cup. The level of extract should be above the bottom of the arrow tape yet below the top of the arrow on the bottom portion of the strip.
7. To prevent cross-contamination, thoroughly clean blender parts and jars of dust and residue prior to preparation of a second sample. Use a new transfer pipette and cup for each sample.



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

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 three-ounce cup containing 12 mL of the liquid soybean extract. 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.

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 "<LOD" (less than the Limit of Detection). 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 Notes

- 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.
- 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 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 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, however, to interpret negative results prior to 5 minutes.
- Protect all components from hot or cold extremes of temperature when not in use.
- CAUTION: Tightly closed containers of soy extract, if left sitting for several hours, may ferment and cause the lid or container to burst. Dispose of extract when testing is complete.

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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EnviroLogix has developed this kit using proprietary reagents.