QuickStix™ Kit for LibertyLink® Rice

Catalog Number AS 013 RB

Highlights:
- *Results in 10 minutes or less*
- *Available as 100-strip kits*

Contents of Kit:
- *100 QuickStix Strips packed in two moisture-resistant canisters*
- *100 Extraction Vials*
- *EB2 Buffer*

Items Not Provided:
- *Oster® Sunbeam blender, 4000 series or equivalent**
- *Ice blade with rubber gasket**
- *½ pint (8 oz.) Mini blender jars**
- *Weigh boats* or weigh paper*
- *Pipette capable of delivering between 1 and 3 mL of buffer**

** Available through EnviroLogix as accessory items; see list on page 3*

Weigh, grind seeds

Transfer crushed seeds to vial

Intended Use

This EnviroLogix QuickStix Kit for LibertyLink Rice is designed to extract and detect PAT/*bar* protein in bulk rice samples. The QuickStix Strips will detect 1.33% Event LL601 rice (i.e. 1 LL601 seed in 75 non-transgenic seeds). Testing multiple 75-seed samples makes it possible to detect 1% or 0.5% contamination with increasing confidence levels. This kit will also detect PAT/*bar* expressed by other types of LibertyLink rice; since the protein is the same, differentiation between them may be confirmed by alternate methods.

How the Test Works

In order to detect the PAT/*bar* protein expressed by LibertyLink rice, the sample must first be extracted in buffer 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.

Selecting a Sampling Protocol

The sampling scheme can dramatically affect the probabilities and level of confidence in detecting any LibertyLink rice that may be commingled in the sample. The chart that follows shows the probability of detecting a given concentration of LL601 rice in the sample, and how the probability of detection is increased with multiple samples.

Table 1 – Number of 75-seed subsamples required

Confidence Level	LL601 Screening Level			
	5%	3%	1%	0.5%
95%	1	1	4	8
99%	1	2	7	13

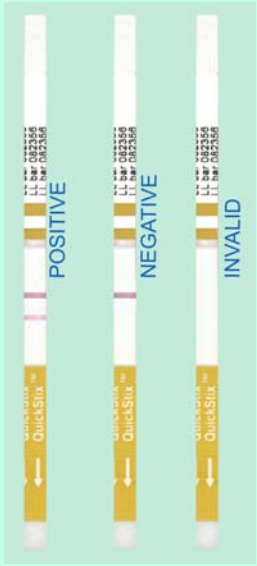
Sample Preparation

This protocol calls for a small sample to be analyzed. It is essential that this sample be well-mixed and representative of the larger bulk. It is the responsibility of the user to ensure proper sampling and thorough mixing prior to analysis. The USDA has published recommended guidelines for obtaining representative grain samples (see list of references on page 3). Once representative samples have been obtained from the truck or container, they can be reduced in size using a splitter and uniformly ground and mixed using an Oster blender or equivalent.

1. Weigh sample and add to the blender jar. Either count 75 seeds and weigh, or determine average weight of seeds to be tested [weigh 100 seeds, divide by 100]; calculate the weight of the sample to be tested [number of seeds x average weight/seed].

Add buffer, cap and shake

Place strip in extraction vial



Any clearly discernable pink Test Line is positive

2. Grind sample for 15-20 seconds on high until all whole grains are finely ground. Empty ground rice in a weigh boat or weigh paper, then carefully transfer to the 5 mL Extraction Vial.
3. Calculate 1.5 times the weight of the sample to determine the volume of buffer. (For example, 75 seeds with a total weight of 1.5 grams would require 2.25 mL buffer.) Use a pipette to accurately add buffer to the vial, then cap and shake for 15 seconds. Tap down solids to bottom of the vial. No further transfer is required; the test will run in the Extraction Vial.

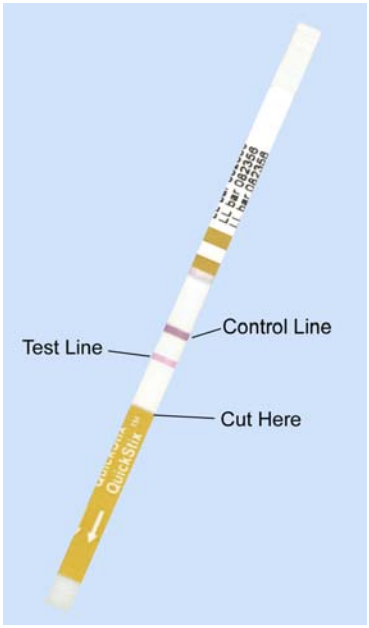
Important: To prevent cross-contamination, thoroughly clean blender parts to remove dust and residue prior to preparation of each sample. Use a new vial for each sample. Do not re-use pipette tips or disposable pipettes.

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 extraction vial. Do not push strip into settled solids. The liquid sample will travel up the strip.
3. Allow the strip to develop for 10 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.

Interpreting the Results

Development of the Control Line within 10 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 sample extract contains PAT/*bar*, a second line (Test Line) will develop on the membrane strip between the Control Line and the protective arrow tape. Any Test Line, no matter how light, should be interpreted as positive for LibertyLink expression.

If no Test Line is observed after 10 minutes, the results should be interpreted as negative. A negative result means the sample contains less than 1.33% LibertyLink rice.

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.

USDA Resources:

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.

Precautions and Limitations

- This kit is designed for screening for presence or absence only and is not meant to be quantitative.
- 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 negative result with this kit does not mean that the sampled grain has not been otherwise genetically modified.
- A strong positive result may safely be interpreted in as little as 5 minutes after sample addition. It is not safe, however, to conclude that a sample is negative before a full 10 minutes has elapsed. A weakly positive sample may require the full 10 minutes for a distinct Test Line to appear.
- Protect all components from hot or cold extremes of temperature when not in use. Do not leave in direct sunlight or in vehicle.

Accessory Items Available

- Oster Sunbeam Blender Model 4094 (Cat. # ACC 044)

- 8 oz. plastic blender jars with caps for Oster Sunbeam (10/pk, Cat. # ACC 046)
- Replacement blade for Oster blender (Cat. # ACC 045)

- Weigh boats (100/pk, ACC 047)
- Pipette and bulb (ACC 048 [5 mL pipette], ACC 049 [bulb])

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