QuickComb™ Kit for Bulk Soybeans

Catalog Number AS 076 TC

Highlights:

- *Results in 5 minutes or less*
- *Convenient comb format*
- *One Common Extraction™:*
  - *Roundup Ready®*
  - *DMO/RR2 Xtend®*
  - *LibertyLink®*

Contents of Kit:

- *100 QuickCombs, packaged 5 combs per foil pouch*
- *Sample cups and disposable transfer pipettes*

Items Not Provided:

- *Blender for sample prep:*
  1. *Oster® Sunbeam blender model #4094 or equivalent (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*
- *Protective cover for blender jar while grinding*

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

This QuickComb Kit for Bulk Soybeans is designed to extract and detect the presence of certain proteins at the levels typically expressed in genetically modified soybeans. The QuickComb may contain the following QuickStix™:

Protein/Trade Name	Sensitivity
CP4 EPSPS / Roundup Ready	1 soybean in 1000 (0.1%)
DMO / Roundup Ready 2 Xtend	1 soybean in 400 (0.25%)
PAT/*pat* / LibertyLink*	1 soybean in 200 (0.5%)*

*The stated detection level is for single-trait LibertyLink LL27 soybean; the higher expression of PAT/*pat* in newer stacked traits may drive the detection level as much as ten-fold lower. For more accurate quantitation of all PAT/*pat*-containing seed stacks, use TotalTrait AQ-076-TCK2367.

How the Test Works

In order to detect the proteins expressed by genetically modified bulk grain, the sample must first be extracted to solubilize the protein. All QuickStix included in the QuickComb are specially formulated to detect their analytes in a Common Extraction.

Each QuickStix Strip has an absorbent pad at each end. The protective tape with the arrow indicates the end of the strips to insert into the reaction cup. The sample will travel up the membrane strips 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 comb.

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 another 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 GMO soybeans in the lot is below the selected purity standard. This calculation should be done for each trait tested, then choose the largest sub-sample volume result.

Step 2: Determine Sub-sample Weight, Jar Size, Grind Times and Water Volume Needed for Sample Preparation

1. Determine the **average weight** of the grain from the lot to be tested. Count and weigh 100 kernels/seeds, then divide by 100.
2. Calculate the sub-sample weight (g) needed for testing, (number of seeds X **average seed weight)**.

USDA References:

- **www.gipsa.usda.gov/fgis/handbook/BK1/BookI_2015-09-18.pdf** - USDA Grain Inspection Handbook, Book 1, Grain Sampling.
- **www.gipsa.usda.gov/fgis/biotech/sample2.htm** - Guidance document entitled Sampling for the Detection of Biotech Grains.
- **www.gipsa.usda.gov/fgis/biotech/sample1.htm** - Practical Application of Sampling for the Detection of Biotech Grains.
- **www.gipsa.usda.gov/fgis/biotech/samplingplan1.xlsx** - 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.



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

3. Calculate water volume needed for sample preparation. The Soybean Common Extraction uses a water volume to sample weight ratio of **5 to 1.**

Example Calculation using a 100 seed sub-sample with an average seed weight of 0.15g.
0.15g x 100 =15 g x 5 mL = 75 mL water for extraction

4. 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). The grind quality should be such that 40-50% passes through a 20-mesh sieve.

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.

Step 3: Prepare the Sample

1. Weigh sample into the appropriate vessel.
2. Put protective cover over glass jars.
3. Grind sample on high speed all whole beans are finely ground.
4. Add the volume of tap water calculated above (see formula above or at left).
5. Cap and shake jar vigorously for 30 seconds, insuring that the entire sample is wet. 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 portion from above the settled sample into the sample cup. The level should be above the bottom of the arrows but below the top of the lower colored portion of the QuickComb strips.
7. To prevent cross-contamination, thoroughly clean blender parts and jars to remove dust and residue prior to preparation of each sample, and use a new sample cup for each. If pipetting, use a new tip or disposable pipette for each sample.

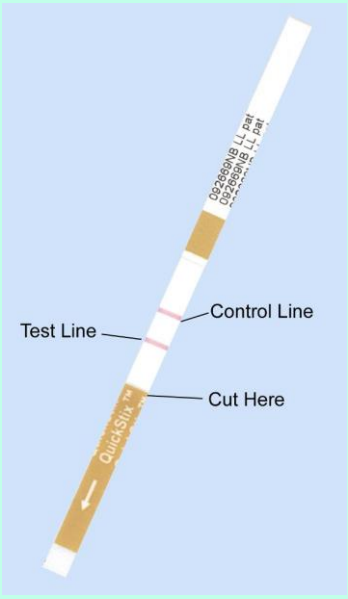
How to Run the QuickComb Test

1. Allow refrigerated foil pouch to come to room temperature before opening. Remove the QuickCombs to be used. Avoid bending the strips or handling the loose comb end. Reseal the canister immediately, if so packaged.
2. Place the comb into the three-ounce cup containing 12 mL of the liquid soybean extract. The sample will travel up the strips.
3. Allow the comb to develop for 5 minutes before making final assay interpretations. Positive sample results may become obvious much more quickly.
4. Remove the QuickComb from the cup to read visually. To retain the combs, cut off and discard the bottom section of each strip covered by the arrow tape.

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



Place QuickComb in cup



Any clearly discernable pink Test Line is considered positive

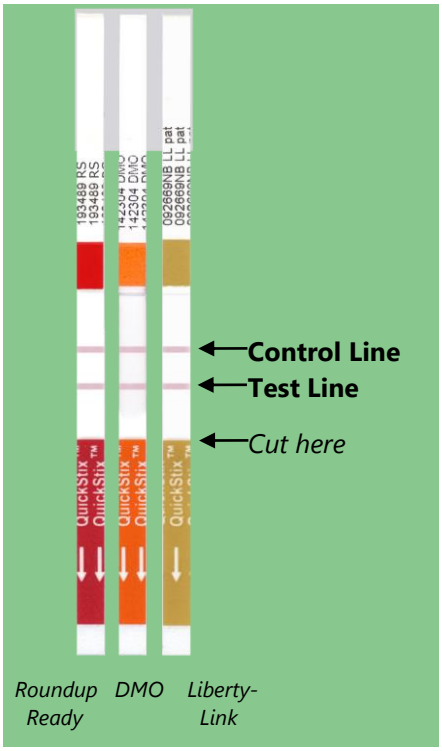


Interpreting the Results

Development of the Control Lines within 5 minutes indicates that the strips have functioned properly. Any strip that does not develop a Control Line should be discarded and the sample re-tested using another comb or corresponding strip.

If the extract is from a sample containing at least the detection level of the strip's analyte on the QuickComb, 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 that strip's protein expression.*

If the extract is from a sample containing less than the listed detection levels, the strip will only develop a Control Line.



Kit Storage

This QuickComb Kit should be stored at room temperature, or refrigerated for longer shelf life. Please note 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. Important: do not open the container (foil pouch or canister) until ready to use the combs. Allow container to come to room temperature before opening to prevent condensation. Immediately re-seal unused QuickCombs in the canister, if so packaged.

Precautions and Notes

- This kit is designed to be read visually as a screen for presence or absence, and is no designed 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 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.
- 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.



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