QuickComb™ Kit for Bulk Grain

Catalog Number AS 036 TC

Highlights:

- *Results in 5 minutes or less*
- *Any combination of strips in convenient comb format*
- *Up to 7 tests - in any combination - from one Common Extraction™:*
  - *Cry1Ab (Bt)*
  - *NK 603 (Roundup Ready®)*
  - *Cry3Bb (YieldGard® Rootworm)*
  - *Cry1F (Herculex™ I)*
  - *T25 (LibertyLink®)*
  - *Cry34 (Herculex RW)*
  - *mCry3A (Agrisure RW)*

Contents of Kit:

- *4 to 8 QuickStix Strips per comb, packaged 5 combs per foil bag (3 strips per comb or less are packaged 10 combs per canister)*
- *Sample cups and disposable transfer pipettes*

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*

USDA References:

Intended Use

This EnviroLogix QuickComb Kit for bulk grain is designed to extract and detect the presence of certain proteins at the levels typically expressed in genetically modified corn bulk grain. The QuickComb may contain any combination of three to seven of the following QuickStix™:

Protein/Trade Name		Sensitivity
Cry1Ab/Bt11, YieldGard Corn Borer	0.8%	~6 kernels in 800
NK 603/Roundup Ready	0.5%	4 kernels in 800
Cry3Bb/YieldGard Rootworm	0.5%	4 kernels in 800
Cry1F/Herculex I	0.5%	4 kernels in 800
T25/LibertyLink	0.5%	4 kernels in 800
Cry34/Herculex RW	0.5%	4 kernels in 800
mCry3A/Agrisure RW	0.9%	~8 kernels in 800

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 vial. 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 corn 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.

   For sampling scenarios for different screening or confidence levels, refer to the USDA/GIPSA Excel spreadsheet described under Step 1 above, or call EnviroLogix Technical Support for assistance.

- **http://www.gipsa.usda.gov/fgis/handbook/gihbk1_inspec.aspx** - USDA Grain Inspection Handbook, Book 1, Grain Sampling.
- **http://www.gipsa.usda.gov/fgis/biotech/sample2.htm** - Guidance document entitled Sampling for the Detection of Biotech Grains.
- **http://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.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.

Corn
Grams of Corn x 1.5=mL of water
For example:
(100 x 0.25)=25g x 1.5=38mL water

Transfer extract to cup, about 2 pipettefuls

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)**.
3. Choose an appropriate jar size for your sample based upon Table 2.
4. Calculate water volume needed for sample preparation. The water volume to sample weight is a ratio of **1.5 to 1.**

Example Calculation using a 100 kernel sub-sample with an average kernel weight of 0.25g.
0.25g x 100 =25 g x 1.5ml =38 mL water for extraction

Table 2

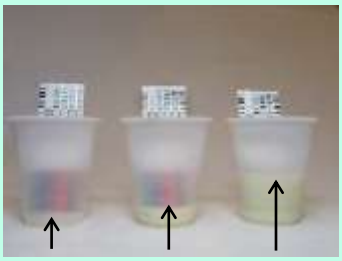
Commodity	Sub-sample Weight (g)	Jar Size (oz.)	Grind Time (sec.)
Corn	10-25	4	30
	25-65	8	30
	65-250	32	45

Step 3: Prepare the Sample

1. Weigh sample into the appropriate size glass Mason jar and attach jar adapter with blade.
2. Place unit on the Waring blender (or equivalent) and cover with protective cover.
3. Grind sample on high speed for specified grinding time or until all whole kernels are broken.
4. Add the volume of tap water calculated by the formula at 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.
6. Transfer 6 to 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. Avoid pulling up particles.
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

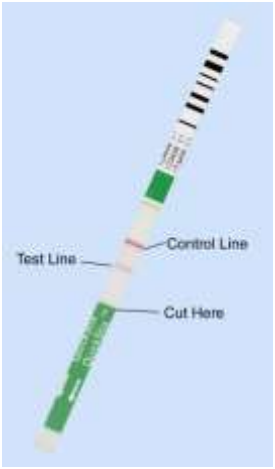
1. Remove a QuickComb from the container (foil bag or canister) and return unused combs to original container (avoid handling loose comb end). Use the blank space on the back of the comb to label sample, if desired. Place the comb of strips into the sample cup, being sure to insert the end indicated by the arrows on the protective tape.
2. After inserting the comb into the extract, liquid will travel up the membrane strips toward the absorbent pads at the top of the strips. Soon after complete wetting of the membranes, lines will appear on the membranes approximately 1/4 inch below the top absorbent pad. This is the Control Line.



too little extract *just right* *too much extract*

Any clearly discernable pink Test Line is considered positive

3. The results should develop within 5 minutes. Allow the strips to develop for a full 5 minutes before making final negative assay interpretations. Strongly positive samples may show results much sooner. Remove the QuickComb from the cup to read. To retain the combs, cut off and discard the bottom section of each strip covered by the arrow tape.



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 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 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. Important: do not open the container (foil bag or canister) until you are ready to use the combs. Allow container to come to room temperature before opening to prevent condensation. Immediately re-seal unused QuickCombs in the container.

Precautions and Notes

- This kit is designed to screen 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 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.

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