ENVIROLOGIX™

# QuickComb™ Kit for QuickScan Bulk Grain

*Catalog Number AQ 036 TC*

***Highlights:***

- *Results in 5 minutes*
- *Any combination of strips in convenient comb format*
- *Up to 8 tests - in any combination - from one Common Extraction™:*
  - *Cry1Ab/Cry1A.105 (Bt)*
  - *Cry9C (StarLink®)*
  - *CP4 EPSPS (Roundup Ready®)*
  - *Cry3Bb (YieldGard® Rootworm)*
  - *Cry1F (Herculex™ I)*
  - *PAT/*pat *(LibertyLink®)*
  - *Cry34 (Herculex RW)*
  - *mCry3A (Agrisure RW)*

***Contents of Kit:***

- *5 to 8 QuickStix Strips per comb, packaged 5 combs per foil bag*
- *Sample cups and disposable transfer pipettes*

***Items Not Provided:***

- *Waring blender, model 31BL91 or equivalent, with glass jar adapter (Eberbach #E8495) and glass Mason jars*
  *~~OR~~*
- *Bunn grinder or equivalent*
- *Graduated cylinder*
- *Tap water*
- *Protective cover for blender jar while grinding*
- *QuickScan System (for quantitative results)*

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 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 five to eight of the following QuickStix™:

| Protein/Trade Name | | Sensitivity |
|---|---|---|
| Cry1Ab-Cry1A.105-Bt11 / YieldGard Corn Borer, and *in* Genuity varieties | 0.8% | ~6 kernels in 800 |
| Cry9C / StarLink | 0.25% | 1 kernel in 800 |
| CP4 EPSPS / 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 |
| PAT/*pat* / LibertyLink | 0.5% | 4 kernels in 800 |
| Cry34 / Herculex RW | 0.5% | 4 kernels in 800 |
| mCry3A / Agrisure RW | 1.0% | 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 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." Results may then be scanned and interpreted quantitatively with the EnviroLogix QuickScan System. 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.


*USDA References:*

- **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.

***Corn Common Extraction***
*Grams of Corn x 1.5=mL of water*

*For example, 400 kernels with an average seed weight of 0.3 g:*

*(400 x 0.3)=120 g of corn*
*120 g x 1.5=180 mL water*

## 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 appropriate container based on sub-sample weight. If using Waring blender, use the table below to choose the appropriate jar size and grinding time.

| Commodity | Sub-sample Weight (g) | "Mason"-type Jar Size (oz.) | Grind Time (sec.) |
|---|---|---|---|
| Corn | 60-120 | 12 | 30 |
| | 120-250 | 32 | 45 |

4. Calculate water volume needed for sample preparation. The Common Extraction Method calls for a water volume to sample weight ratio of **1.5 to 1.**

*Example Calculation using a 400 kernel sub-sample with an average kernel weight of 0.3g.*
*0.3g x 400 =120g x 1.5mL =180 mL water for extraction*

## Step 3: Prepare the Sample

***Bunn grinder or equivalent:***

1. Weigh out subsample based on the average weight per seed calculation.
2. Grind subsample (using Auto-Drip setting if using a Bunn grinder) with grinder until all whole grains are broken. The sample should be the consistency of coffee grounds.
3. Place subsample into an appropriately sized jar or zip-type plastic bag and add the volume of tap water calculated using the Corn Common Extraction formula (left).
4. Cap the jar or "zip" plastic bag and shake 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. If intending to read the test using QuickScan, be sure to shake for at least 30 seconds, and for accurate results, allow sample to settle for another 30 seconds.

***Waring blender or equivalent:***

1. Weigh sample into the appropriate size glass Mason jar and attach jar adapter with blade.
2. Place assembly on the Waring blender (or equivalent). Shield with protective cover to prevent injury in the event of jar breakage. Grind sample at high speed for 30-45 seconds, or until all whole grains are broken. The sample should be the consistency of coffee grounds.
3. Add the volume of tap water calculated using the Corn Common Extraction formula (left).
4. Cap the jar and shake 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. If intending to read the test using QuickScan, be sure to shake for at least 30 seconds, and for accurate results, allow sample to settle for another 30 seconds.

5. Transfer 20 mL of the liquid portion from above the settled sample into the sample cup. Pour extract into cup to the 20 mL line, or use a fresh pipette from the kit to transfer extract until the 20 mL line is reached. **Important:** Avoid transferring particles as much as possible, and after transfer, allow the liquid in the sample cup to settle for 30 seconds so that any particles will settle at the bottom of the cup.

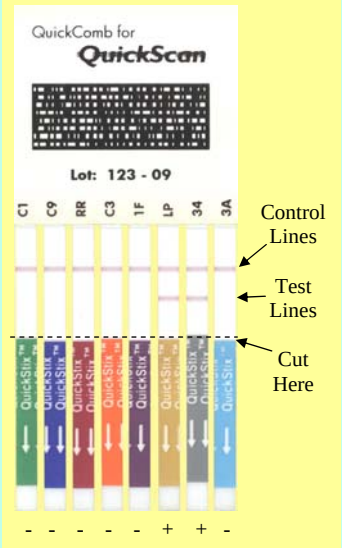
*Transfer 20 mL extract to cup:*

*Either pour…*

*…or pipette to the 20 mL mark*

*(outlined to demonstrate cup size and markings)*

*After 30 seconds, add QuickComb to cup*




*Any clearly discernable pink Test Line is considered positive*

6. 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, always use a new pipette for each sample.

# How to Run the QuickComb Test

1. Remove a QuickComb from the foil bag 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.
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 visually. To retain the combs, or for use in the QuickScan System, cut off and discard the bottom section of each strip covered by the arrow tape. If reading QuickComb using QuickScan, remember that combs must be read immediately after cutting, while still wet.

# 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 target protein.*

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

Results may then be scanned and interpreted quantitatively with the QuickScan System. Please consult the QuickScan User Manual for details.

# 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 foil bag until ready to use the combs. Allow container to come to room temperature before opening to prevent condensation.

# 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.
- 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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