

Contents of Kit:

- 9 QuickStix Strips per comb, 100 combs packaged 5 per foil bag
- 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

Catalog Number AS 036 TC

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 contains nine QuickStix™:

| Protein/Trade Name | Sensitivity | |
|--|-------------|-------------------|
| Cry1A (MON810) / YieldGard Corn Borer* | 0.8% | ~6 kernels 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 | 0.9% | ~8 kernels in 800 |
| Cry2A / in SmartStax (MON89034) | 0.9% | 8 kernels in 800 |
| Vip3A / Viptera | 0.25% | 2 kernels in 800 |
| * The Cry1A strip will also detect Cry1A.105 and Bt11, but the detection level is based on MON810. | | |

How the Test Works

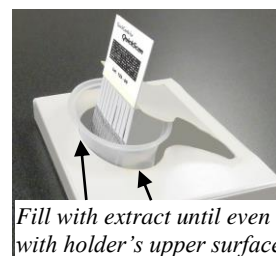
In order to detect the proteins expressed by genetically modified bulk grain, the sample must first be extracted to solubilize the protein. All QuickStix included in the QuickComb are specially formulated to detect their analytes in a Common Extraction.

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 are then scanned and interpreted quantitatively with the EnviroLogix QuickScan System. Please avoid bending the comb.

Sample Preparation

1. Collect a composite sample according to USDA/GIPSA instructions found in the reference documents listed in Precautions and Notes.
2. Determine the **average weight** of the grain from the lot to be tested. Count and weigh 100 kernels/seeds, then divide by 100. Corn kernels vary widely from region to region and from year to year. Be sure to test the desired number of kernels by checking average kernel weight periodically.
3. Calculate the sub-sample weight (g) needed for testing, (number of seeds X **average seed weight**). A sub-sample size of at least 200 grams is required to create enough extract for the test.
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** (see example, right).
5. Weigh sample into a 32-ounce glass Mason jar and attach jar adapter with blade.
6. Place unit on the Waring blender (or equivalent) and cover with protective cover.
7. Grind sample on high speed for 45 seconds or until all whole kernels are broken.
8. Add the volume of tap water calculated by the formula at left.
9. 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.
10. Assemble cardboard holder and lift the QuickComb support. (Note: save and reuse holders.) On a flat surface, insert sample cup in the space provided, then transfer approximately 35 mL of the liquid portion from above the settled sample into the sample cup. Use a fresh pipette from the kit, or pour slowly and carefully, until the depth of the sample is level with the upper surface of the cardboard holder. Important: Avoid transferring particles as much as possible.
11. 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.

Corn Common Extraction™
g of corn X 1.5 = mL of water
Example:
800 kernel sub-sample with an
average kernel weight of 0.3g.
 $0.3 \text{ g} \times 800 = 240 \text{ g} \dots$
 $240 \times 1.5 = 360 \text{ mL water}$



Fill with extract until even
with holder's upper surface

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 QuickComb into the sample cup, using the comb support to hold the it upright, and 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. These are the Control Lines.
3. Allow the strips to develop for a full 5 minutes before making final assay interpretations (positive results may appear before the full 5 minutes are up). 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 device.

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.*

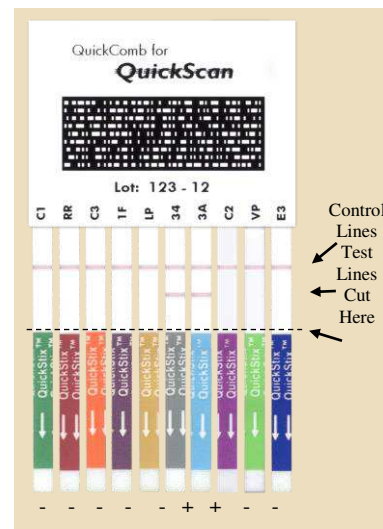
If the extract is from a sample containing less than the listed detection levels, the strip will only develop a Control Line.

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 foil bag until ready to use the combs. Allow container to come to room temperature before opening to prevent condensation. Immediately return unused QuickCombs to foil pouch and close securely.

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.
- USDA References:
 - www.gipsa.usda.gov/fgis/handbook/BK1/Book1_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.



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License

EnviroLogix has developed this kit using proprietary reagents as well as reagents licensed from Monsanto Company.

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