QuickTox™ Kit for Aflatoxin Bulk Grain

Catalog Number AS 101 BG

Intended Use

This EnviroLogix QuickTox Kit for Aflatoxin is designed to quickly extract and screen corn for the presence of total aflatoxins. The QuickTox Kit is designed to provide a qualitative screen for aflatoxin residues at a 20 ppb cut off level in corn grain.

How the Test Works

A composite corn sample is first collected, then extracted to solubilize any aflatoxin present. Each sample should be ground to a fineness of 20 mesh and extracted with diluted solvent. This extract is further diluted for testing with the QuickTox Kit.

Each QuickTox Strip has an absorbent pad at each end. The protective tape with the arrow indicates which end of the strip to insert into the reaction vial. The sample extract travels up the membrane strip and is 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."

Preparation of the Sample

Step 1: Determine Number and Size of Sub-samples

1. Collect a composite corn sample according to your own sampling plan or USDA/GIPSA guidelines. Consult USDA/GIPSA reference documents such as **http://archive.gipsa.usda.gov/reference-library/handbooks/grain-insp/grbook1/bk1.pdf** to help design a plan that fits your needs.
2. Grind samples using a mill which provides a sample that passes through a 20 mesh sieve. Mix ground material thoroughly before sub-sampling.

Step 2: Extract corn sample

1. Weigh 10-50 grams of milled sample into a disposable sample cup with lid and add two volumes of solvent, either 70% methanol or 50% ethanol (2 mL per gram of sample, i.e. 10 grams, add 20 mL).

   70% Methanol Preparation: *For 100 mL, measure 70 mL 100% methanol [reagent grade or better], pour into suitable container with cap.* ***Add*** *30 mL deionized or distilled water. Cap tightly and shake to mix. Use care when uncapping. Methanol can also be purchased at 70% concentration.*

   50% Ethanol Preparation: *For 100 mL, measure 50 mL 100% ethanol [reagent grade or better], pour into suitable container with cap.* ***Add*** *50 mL deionized or distilled water. Cap tightly and shake to mix. Use care when uncapping. Ethanol can also be purchased at 50% concentration.*

   NOTE: For GIPSA testing, a 50 gram sub-sample should be blended with 100 mL of solvent.

2. Cap sample cup tightly and place on shaker for 1 minute. Shaker should be operated at the highest speed. Alternately, samples may be shaken by hand for 1½ to 2 minutes.

Highlights:

- *Negative results in 2-3 minutes*
- *Positive results in only 5 minutes*
- *Simple protocol*

Contents of Kit:

- *50 QuickTox Strips packed in a moisture-resistant canister*
- *50 fixed volume transfer pipettes*
- *50 reaction vials*
- *Available in 50-strip individual kit format or bulk packaging*

Items Not Provided:

- *Orbital/rotary shaker*
- *Plastic sample cups with lids**
- *Solvent (70% methanol or 50% ethanol)**
- *20 mesh screen*
- *Graduated cylinder**
- *Tap water*
- *Timer*

**Available as accessories – see list on Page 3*

Correct 20 mesh grind for corn

Measure solvent

Shake mechanically or by hand

Add water to vial

Add extract to vial

Place strip in vial

3. Sample will immediately begin to separate into 2 layers. The top (yellowish) layer containing the aflatoxin residues will be used in testing.

Step 3: Dilute corn extract with water

1. Using the fixed volume pipette provided, place 150 microliters (150 µL) water into a reaction vial.
2. Using the same fixed volume pipette, remove 150 µL from the top (yellowish) layer of sample. Add extract to reaction vial containing water.
3. Mix water and sample extract thoroughly by stirring with the tip of the fixed volume pipette.

NOTE: To ensure correct volumes are used to prepare the test sample, a fixed volume pipette is included with the kit. When a liquid drawn to the top of the straw end of the pipette is dispensed, 150 µL will be expelled into the reaction vial. Any overfill is retained in the pipette. After diluting the sample, the final volume in the reaction vial should be 300 µL. Do not **reuse** diluted samples. Use a new fixed volume pipette and reaction vial for each sample.

How to Run the QuickTox Strip Test

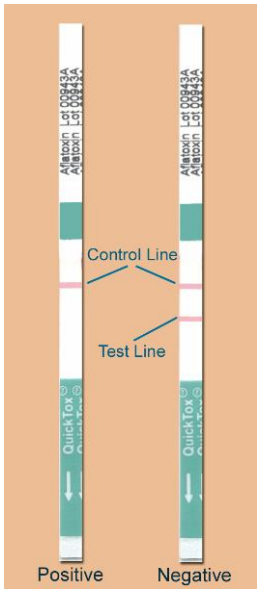
1. Allow refrigerated canisters to come to room temperature before opening. Remove the QuickTox Strips to be used. Avoid bending the strips. Reseal the canister immediately.
2. Place the strip into the reaction vial containing the diluted sample extract. The arrow tape on the end of the strip should point into the reaction vial.
3. The sample extract will travel up the strip (flow may not be visible immediately—this is expected and normal). Reaction vials will stand on their own or may be inserted into the cardboard rack provided.
4. Allow the strip to develop for 5 minutes before making final assay interpretations. Negative sample results may become obvious much more quickly (2-3 minutes).
5. To retain the strip, cut off and discard the bottom section of the strip covered by the arrow tape.

Interpreting the Results

Development of a Control Line within 5 minutes indicates that the strip has functioned properly. Any strip that does not develop a Control Line should be discarded. A second preparation of the extract (using a fresh 1:2 dilution) should be made and tested using another strip.

Negative Results – A sample containing aflatoxin residues of less than 10 ppb will develop **2 distinct lines** in the test area. A negative test result can be interpreted as soon as a Test Line develops, generally <u>within 2-3 minutes</u>.

Positive Results – The QuickTox Kit for Aflatoxin is designed to screen for aflatoxin at levels of approximately 20 ppb or higher in corn grain. A sample containing aflatoxin residues of 20 ppb or higher will develop **1 distinct line**, the Control Line. **The absence of a Test Line should be interpreted as positive for aflatoxin residues**. Allow the strip to develop for the full <u>5 minutes</u> before concluding that the sample has tested positive for aflatoxin.



Optional Items Available:

- Negative Ground Corn Sample
- Positive Ground Corn Sample
- Sample cups with lids
- Graduated cylinder
- 70% Methanol
- 50% Ethanol

Some samples containing slightly less than 20 ppb may also provide a positive result. You may wish to confirm positive results with a quantitative method to determine the precise level of contamination.

Kit Storage

This QuickTox Kit should be stored refrigerated. Note the shelf life on the kit box. 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 strips.

Cross-reactivity

The following mycotoxins have been tested with this kit using the protocols specified herein. No false positive results occurred at the tested levels (concentration in starting material).

- DON (deoxynivalenol) - 200 ppm
- Ochratoxin A – 200 ppm
- Fumonisin B_1 – 200 ppm

Precautions and Notes

- This kit is designed to screen for presence or absence only, and is not designed to be quantitative.
- This product is currently not applicable for use in testing any other crops.
- As with all screening tests, it is recommended that results be confirmed by an alternate method when necessary.
- The assay has been optimized for use 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 diagnostic tool 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 may safely be interpreted in as little as 2-3 minutes after beginning the test. It is not safe, however, to interpret positive 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 vehicle.
- For convenience, controls and solvent can be ordered through EnviroLogix (see list, left).
- **IMPORTANT:** Methanol and ethanol are flammable and toxic. Avoid inhaling vapors or contact with the skin, eyes, or clothing. Wear personal protective equipment including safety glasses, nitrile gloves (**not latex**), a vapor mask and a lab coat when handling. Keep containers tightly closed and away from heat, sparks and open flame. Observe any applicable regulations when disposing of samples and kit reagents.
- Liquids containing aflatoxin should be treated by the addition of bleach (add a minimum of 10% of the total volume for 10 minutes). All labware should be soaked for 1 hour or more in a 30% solution of household bleach.

For Technical Support Contact Us At:

EnviroLogix
500 Riverside Industrial Parkway
Portland, ME 04103-1486
USA
Tel: (207) 797-0300
Toll Free: 866-408-4597
Fax: (207) 797-7533

e-mail:
info@envirologix.com

website:
www.envirologix.com

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License

EnviroLogix has developed this kit using proprietary reagents.