United States Department of Agriculture
Grain Inspection, Packers and Stockyards Administration
Federal Grain Inspection Service

Program Notice

FGIS-PN-14-03 8/13/14

ENVIROLOGIX QUICKTOX FOR QUICKSCAN AFLATOXIN FREE QUANTITATIVE TEST KIT

1. PURPOSE

The purpose of this program notice is to provide test instructions for the QuickTox Kit for QuickScan Aflatoxin FREE test method approved by the Technology and Science Division (TSD) for official services. The QuickTox Kit QuickScan Aflatoxin FREE test method, Product Number AQ 209 BG, uses lateral flow strip assay technology, and a non-hazardous (water-based) extraction powder that eliminates the use of organic solvents (methanol, ethanol, acetonitrile, etc.) to report aflatoxin results in parts per billion (ppb) in corn, and wheat.

2. BACKGROUND

The TSD provides performance evaluations of mycotoxin test kits for manufacturers seeking approval of their product for use in the official inspection system. The Policies, Procedures, and Market Analysis Branch (PPMAB), provides instructions for the use of approved mycotoxin test kits. In an effort to offer official use of the EnviroLogix QuickTox for QuickScan Aflatoxin FREE test method in a timelier manner, PPMAB has decided to issue this program notice prior to the release of the revised Mycotoxin Handbook. The following test procedures are approved for use by official testing locations.

3. TEST PROCEDURES

a. General Information.

The QuickTox Kit for QuickScan Aflatoxin FREE test method provided by the EnviroLogix Corporation is a single-step lateral flow immunochromatographic assay based on a competitive immunoassay format. The test provides quantitative analysis for the presence of aflatoxins, using a non-hazardous, water-based extraction solution along with an aflatoxin-antibody particle complex coated test strip and EnviroLogix QuickScan System. Following approved test instructions and EnviroLogix's assay principles the inspector can quantitatively determine the presence of aflatoxins in a sample.

The test kit is limited to providing quantitative aflatoxin measurements 5 to 30 parts per billion (ppb) using standard assay and up to 100 ppb with an additional dilution procedure. The maximum reporting limit is 100 ppb.

Obtain samples according to the instructions in the Grain Inspection Handbook, Book I "Grain Sampling," and refer to the Aflatoxin Handbook Chapter 3 "Sample Preparation for minimum sample requirement and sample preparation procedures."

Federal Grain Inspection Service (FGIS) employees must comply with all applicable safety and sanitation requirements as listed in this program notice to ensure a safe and efficient work environment. Refer to all Federal, (*Environmental Protection Agency, Title 40 CFR*), State, and local laws and regulations applicable to the management of hazardous waste.

<table>
<tr><th colspan="6">Approved Test Kit Information</th></tr>
<tr><td>Test Kit Vendor:</td><td colspan="5">EnviroLogix Inc. 1-207-797-0300</td></tr>
<tr><td>Test Kit Name:</td><td colspan="5">EnviroLogix QuickTox Kit for QuickScan Aflatoxin FREE</td></tr>
<tr><th>Product Number</th><th colspan="2">Conformance Limits</th><th>Type of Service</th><th>Temp Range</th><th>Supplemental Analysis</th></tr>
<tr><td>AQ 209 BG</td><td>Min</td><td>Max</td><td rowspan="3">Quantitative</td><td rowspan="3">64-86° F</td><td rowspan="3">No</td></tr>
<tr><td>FOL Code</td><td rowspan="2">5 ppb</td><td rowspan="2">100 ppb</td></tr>
<tr><td>AFLJB</td></tr>
<tr><td colspan="2">Grain/Commodities Approved for</td><td colspan="4">Corn and wheat.</td></tr>
<tr><td>Test Format:</td><td colspan="5">Single-Step Lateral Flow Strip.</td></tr>
<tr><td>Extraction Method:</td><td colspan="5">Shake 50-gram sample with two packets of EB17 extraction powder and 150 milliliters (mL) of distilled or deionized water for 1 minute using an orbital shaker at high speed (~280 rpm), by hand or mechanical shaker with similar shaking motion.</td></tr>
<tr><td>Detection Technique:</td><td colspan="5">Envirologix QuickScan System.</td></tr>
</table>

b. <u>Extraction procedures for corn</u>.

(1) Transfer 50 grams (+/- 0.2 g) of ground sample into an extraction mixing jar/vessel.

(2) Add the contents of 2 extraction packages of EB17 to the 50 gram corn sample and (shake by hand) to evenly distribute the extraction buffer components throughout the sample.

(3) Add 150 mL of distilled or deionized water and seal the extraction container.

(4) Cover the extraction jar and shake for **1 minute** using an orbital shaker at high speed (~280 rpm), by hand or mechanical shaker with similar shaking motion.

Shake mechanically

(5) Filter the sample through an approved coffee filter (EnviroLogix part no. 11434) into a clean collection vessel. This is the **filtered sample extra**ct for testing.

Filter extract with an approved coffee filter

(6) Proceed to **Test Procedures.**

c. <u>Extraction Procedures for wheat</u>.

(1) Transfer 50 grams (+/- 0.2) of ground sample into an extraction mixing jar.

(2) Add 150 mL of distilled/deionized water and seal the extraction container and wet wheat thoroughly before adding the contents of 2 extraction packages of EB17.

(3) Cover the extraction jar and shake for **1 minute** using an orbital shaker at high speed (~280 rpm), by hand or mechanical shaker with similar shaking motion.

Shake mechanically

(4) Fill a micro-centrifuge tube with extract. Centrifuge for 30 seconds at

2000 x g (not rpm). The top layer containing the aflatoxin residues will be used for testing. This is the **centrifuged sample extract** for testing.

Centrifuge 30 seconds at 2000 x g

(5) Proceed to **Test Procedure**s.

d. Test Procedures.

(1) Sample Preparation and Analysis for 5 to 30 ppb quantitation range.

(a) Using a calibrated pipette with new pipet tip, place 100 microliters (µL) of assay buffer DB5into a reaction vial. Take care not to contaminate buffer. Use a new tip for each vial containing buffer.

(b) Using a new pipette tip and avoiding particulates, add 100 µL of filtered sample extract (i.e., top layer of the centrifuged sample extract) to the reaction vial containing buffer.

(c) Mix buffer and sample extract thoroughly by pipetting up and down 5 times with the pipette tip.

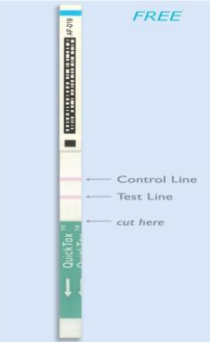
NOTE: Samples that are not thoroughly mixed and/or accurately pipetted will adversely affect test results. After diluting the sample, the final volume in the reaction vial should be 200 µL. Do not reuse diluted samples. Use a new reaction vial for each sample. Use two pipette tips (one for buffer, one for extract) for each sample.

(d) Place an assay test strip into the vial with the arrow end down.

Place strip in vial

(e) Allow the test strip to develop for **4 minutes for corn, and 5 minutes for wheat.**

(f) After designated incubation immediately cut off the arrow end of the strip.



Cut strip and place in QuickScan reader immediately —no drying step!

(g) Place the test strip face down in the carrier with the barcoded end closet to the handle.

(h) Push the Read Test button.

(i) Results are displayed; ensure the pull down tab indicates 1:1.

(j) Make the appropriate sample notations on the results screen window and work record.

(k) Results are archived in the Datalog file.

Note: See Interpreting the Lateral Flow Test Strip with QuickScan Reader section for information.

(2) <u>Sample Preparation and Additional Dilution Procedure for 30 to 100 ppb quantitation range.</u>

(a) Prepare Dilution Solution by dissolving one EB17 packet in 300 mL of distilled/deionized water. Label, date and document technician prepared by. (This is not a clear solution and may be stored at ambient temperature for 30 days). Thoroughly mix before use.

(b) Using a calibrated pipette with a new pipette tip, add 500 µL of EB17 solution to a clean micro-centrifuge tube.

(c) Using a new pipette tip, add 100 µL of **filtered sample extrac**t to the 500 µL of EB17 solution and mix by vortexing 5 seconds. This is the Diluted Sample Extract for testing.

(d) Add 100 µL of assay buffer DB5 to a new reaction vial.

(e) Add 100 ul of the Diluted Sample Extract sample to the reaction vial and mix by pipetting up and down 5 times.

(f) Place as assay strip into the reaction vial with the arrow end down.

(g) Allow the strip to develop for **4 minutes for corn and 5 minutes for wheat**. Remove after development time and cut-off the arrow end of the strip.

(h) Place the test strip face down in the carrier with the barcoded end closet to the handle.

(i) Push the Read test button.

(j) Results are displayed; ensure the pull down tab indicates 1:6

Note: Choose 1:6 under the dilution pull down tab on QuickScan Results Screen to obtain results. The system will calculate the 6 fold dilution factor using the drop down menu.

(k) Make the appropriate sample notation(s) on the results screen window and work record.

(l) Results are archived in the Datalog file.

Note: Official locations may initially use the Additional Dilution Procedure per applicant request to report test results above 30 ppb. <u>Do not report test results below 30 ppb using the Additional Dilution Procedure.</u>

e. <u>Interpreting the Lateral Flow Test Strip with QuickScan Reader.</u>

Development of a Control Line within stated development times 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 buffer dilution) should be made and tested using another test strip.

(1) Place the test strip face down in the carrier with the barcoded end closet to the handle.

(2) Insert the carrier into the reader and the strips are read by clicking on the "Read Test" area of the screen.

Note: For quantitation between 30 to 100 ppb choose 1:6 under the dilution tab on QuickScan Results Screen. The system will calculate and record the aflatoxin level of the diluted samples.

f. Reporting and Certifying Test Results.

(1) Report all results on the pan ticket and inspection log to the nearest whole ppb.

(2) Sample results over 30 ppb are reported as greater than 30 ppb (> 30 ppb) for corn and wheat. Upon applicant request, the additional dilution procedure may be performed for reporting results above 30 ppb.

(3) Analysis results that are above 100 ppb are reported as exceeding the limit (i.e., > 100 ppb).

(4) Refer to the Aflatoxin Handbook, Chapter 4, Certification, for more detailed certification statements and procedures.

g. Cleaning Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge labware, wash thoroughly, and then rinse with a copious amount of clean water before reusing.

h. Waste Disposal.

The remaining extract and disposable materials are considered non-regulated hazardous waste by current federal regulations and suitable for normal/routine disposal. Please review State and local regulations which may be more stringent to ensure compliance.

i. Equipment and Supplies.

(1) Materials Supplied in Test Kits.

(a) QuickTox QuickScan™ System. (ACC131)

(2) Materials Required but not Provided:

(a) Mini-Pet pipette. (ACC041) –(*one location free)*

(b) Sample cups with lids. (ACC 012-50)

(c) Distilled or Deionized water.

(d) Balance, Scale.

(e) Sample Grinder.

(f) Graduated cylinder. (150 – 200 ml)

(g) Orbital/rotary shaker.

(h) Micro-centrifuge. (ACC 064 E)

(i) Dilution set (extra tips and vials for 100 dilutions for test samples above 30ppb). (ACC 080)

(j) 50 gram sample Extraction, set additional packets and sample extractions bags. (ACC035)

(k) Dilution Set: Disposables and Extraction Powder for 100 dilutions. (ACC034)

(l) Centrifugation Set: Disposables for 50 tests. (ACC 010)

(m) Negative Ground Corn Sample 25 grams. (CON-110)

(n) Positive Ground Corn sample 25 grams. (CON-110-P)

j. Storage Conditions.

(1) Test kits should be refrigerated between 36°- 48°F. Prolonged exposure to high temperatures may adversely affect the test results. Do not open the desiccated canister until ready to use the strips.

k. Precautions.

(1) Strips must be read wet promptly at four (4) minutes **corn** or five (5) minutes **wheat** to ensure accurate results.

(2) Do not treat either the liquid extracts or the extraction labware with a bleach solution. The Extraction Packet powder is incompatible with strong oxidizers.

(3) Follow your facility's safety procedures for disposal of samples and extracts potentially containing or known to contain aflatoxin(s).

(4) This product is currently not officially approved for use in testing any other crops beyond bulk corn or wheat.

(5) The assay has been optimized for use with the protocols provided in the kit. Deviation from these protocols may invalidate the results of the test.

(6) Proper and thorough mixing, along with accurate pipetting, are essential to accurate results.

(7) Protect all components from hot or cold extremes of temperature when not in use. Do not leave in direct sunlight or in vehicle.

(8) **IMPORTANT:** Extraction Powder should be considered flammable and an irritant (MSDS available at www.envirologix.com/MSDS-AQ209.pdf). Avoid inhaling powder or contact with the skin, eyes, or clothing. Wear personal protective equipment including safety glasses, gloves, mask, and a lab coat when handling.

(9) Keep powder away from heat, sparks and open flame.

(10) Observe any applicable regulations when disposing of samples and kit reagents. Do not use the test kits beyond the noted expiration date.

4. FILING

Retain a copy of this program notice until the Mycotoxin Handbook is revised to include the test procedures stated herein.

5. QUESTIONS

Direct any questions concerning this program notice to Carl Jackson, Policies, Procedures, and Market Analysis Branch(PPMAB), at (202) 720-8286, or email at carl.jackson@usda.gov or Patrick McCluskey, PPMAB, at (816) 659-8403, or email at patrick.j.mccluskey@usda.gov.

/s/Robert Lijewski

Robert Lijewski, Director
Field Management Division