ENVIROLOGIX QUICKTOX KIT FOR QUICKSCAN AFLATOXIN FREE QUANTITATIVE AFLATOXIN TEST KIT

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GENERAL INFORMATION

The QuickTox Kit for QuickScan Aflatoxin FREE test method uses lateral flow strip assay technology, and a non-hazardous (water-based) extraction powder procedure that eliminates the use of organic solvents (methanol, ethanol, acetonitrile, etc.) to report aflatoxin results in parts per billion (ppb) in corn.

The instructions presented in this document cover only the procedure for performing the analytical test for official inspections. For questions regarding this procedure, contact Dr. Ajit Ghosh of the Technology and Science Division by phone at 816-891-0417 or email at Ajit.K.Ghosh@usda.gov.

Refer to the Mycotoxin Handbook for information on use of this test kit in official inspections including sampling, general sample preparation reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey of PPMB by phone at 816-659-8403 or email at Patrick.J.McCluskey@udsa.gov.

Approved Test Kit Information

Test Kit Vendor:	EnviroLogix Inc. 1-207-797-0300
Test Kit Name:	EnviroLogix QuickTox Kit for QuickScan Aflatoxin FREE
Product Number:	AQ 209 BG
Effective Date of Instructions:	08/08/2016
Instructions Revision Number:	0
Conformance Range:	5 – 100 ppb
Number of Analyses to Cover Conformance Range:	2
Type of Service:	Quantitative
Approved Commodities:	Corn
Extraction method:	Shake a 50-gram sample with the contents of 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 (280-300 rpm).
Test Format:	Lateral Flow Strip
Detection Method:	QuickScan System (Software Version 4.9.4 Update 1)

PREPARATION OF MATERIALS AND EQUIPMENT

Bring all kit components to room temperature prior use.

Scanner Setup

Scan the Multi-Matrix Barcode Card (MMBC) prior to using a new lot of test strips. Scanning the MMBC is only required once per kit lot. If you plan to test only matrices within the MG1 group (Corn), scan the side of the MMBC card that has only the MG1 barcode. The additional MG barcodes are for non-approved matrices, and if they are scanned, the QuickScan software will prompt users to select a Matrix Group (MG) when strips are scanned.

QuickScan software version 4.9.4 update 1 or higher is needed.

Dilution Solution Preparation

The Dilution Solution is used with the 30-100 ppb Quantitation Range procedure. Prepare Dilution Solution by dissolving one EB17 packet in 300 mL of distilled/deionized water. Mix for approximately 10 minutes using a magnetic stir plate and bar. The solution will remain cloudy.

Label, date, and document the preparation. This solution can be stored at ambient temperature for 30 days. Thoroughly mix before each use. The Dilution Solution is not a clear solution.

EXTRACTION PROCEDURES

Extraction Procedure for Corn

(1) Transfer 50.0 ± 0.2 g of ground sample into an extraction bag.

(2) Add the contents of two EB17 Extraction Powder packets to the sample, and shake by hand to evenly distribute the powdered buffer components throughout the sample.

(3) Add 150 mL of distilled or deionized water and seal the extraction bag. Shake and massage the bag to ensure that the sample is fully wetted and to break up any clumps.

(4) Shake the extraction bag at high speed (280-300 rpm) on an orbital shaker for 1 minute.

(5) Filter the sample through an approved coffee filter (EnviroLogix Part No. 11434) into a clean collection vessel. This is the **clarified sample extract** for testing. It can be used for up to 3 hours.

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

TEST PROCEDURES

a. Sample Preparation and Analysis for 5 to 30 ppb Quantitation Range for Corn

(1) Using a calibrated pipette with new tip, add 100 microliters (µL) of the provided dilution buffer DB5 to a reaction vial.

NOTE: Take care not to contaminate the buffer. Use a **new tip** for each vial containing buffer.

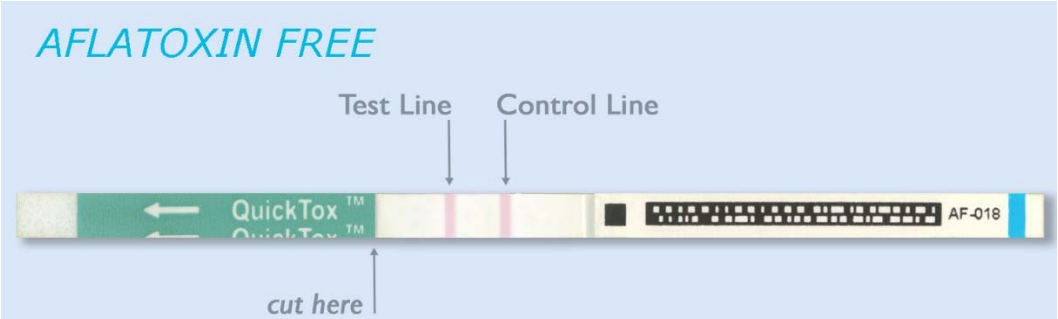
(2) Using a new pipette tip, add 100 µL of **clarified sample extract** to the reaction vial containing the 100 µL of buffer.

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

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

(5) Allow the test strip to develop for **4 minutes.**

(6) After designated incubation time, immediately cut off the entire green portion (arrow end) of the strip.



(7) Promptly place the test strip face down in the carrier with the barcoded end closest to the handle and slide carrier into the reader.

(8) Click the Read Test button.

NOTE: If all the MMBC barcodes were scanned in, a pop-up menu will prompt you to select the Matrix Group that corresponds to the sample type run. Select AF MG1 for corn. MG1 will be automatically selected if only the MG1 barcode was scanned.

(9) On the Results screen, the Dilution level defaults to the 1:1 selection and should not be changed. Results are displayed.

(10) Make the appropriate sample notations on the results screen window and work record. When you click Close, the results and any added notations are stored in the DataLog.

(11) If the result is >30 ppb, re-test the extract using the Sample Preparation and Additional Dilution Procedure for 30-100 ppb Quantitation Range.

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

b. Sample Preparation and Analysis for 30 to 100 ppb Quantitation Range for corn

(1) If not previously prepared, make Dilution Solution as indicated in PREPARATION OF MATERIALS AND EQUIPMENT section above.

(2) Using a calibrated pipette with a new pipette tip, add 500 µL of **Dilution Solution** to a clean micro-centrifuge tube.

(3) Using a new pipette tip, add 100 µL of **clarified sample extract** to the 500 µL Dilution Solution and mix by pipetting up and down 5 times. This is the **diluted sample extract** for testing.

(4) Using a new pipette tip, add 100 µL of assay buffer DB5 to a new reaction vial.

(5) Using a new pipette tip, add 100 µL of the **diluted sample extract** to the reaction vial containing the 100 µL of buffer. Mix by pipetting up and down 5 times.

(6) Place an assay strip into the reaction vial with the arrow end down.

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

(8) Place the test strip face down in the carrier with the barcoded end closest to the handle and slide carrier into the reader.

(9) Push the Read Test button.

(10) If all of the MMBC barcodes were scanned in, a pop-up menu will prompt you to select the Matrix Group that corresponds to the sample type run. Select AF MG1 for corn. MG1 will be automatically selected if only the MG1 barcode was scanned.

(11) On the Results screen, use the drop down menu under the Dilution Column to select 1:A. Results are displayed.

(12) Make the appropriate sample notations on the results screen window and work record. When you click Close, the results and any added notations are stored in the DataLog
Note: Report only values between 30ppb and 100ppb using this 30-100ppb Quantitation Range Procedure.

Interpreting the Lateral Flow Test Strip with QuickScan System.

Development of a Control Line within stated development times indicates that the strip has functioned properly. Strips that do not develop a Control Line are considered invalid and should be discarded and the test repeated. For any strip that does not develop a Control Line, the scanner will report an error code and no results will be displayed.

REPORTING AND CERTIFYING TEST RESULTS

Refer to the Mycotoxin Handbook for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-659-8403 or Patrick.J.McCluskey@udsa.gov).

STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions

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.

b. Precautions

(1) Strips must be read wet, promptly at four (4) minutes to ensure accurate results.

(2) Do not treat either the liquid extracts or the extraction labware with a bleach solution. The EB17 Extraction 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 corn.

(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:** The EB17 Extraction Powder should be considered flammable and an irritant (MSDS available at http://www.envirologix.com/wp-content/uploads/2015/06/MSDS-AQ209.pdf). **Avoid inhaling powder** or contact

with the skin, eyes, or clothing and use in the fume hood. 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.

EQUIPMENT AND SUPPLIES

(Catalog No. shown in parentheses)

a. Materials Required But Not Provided

(1) QuickScan System (ACC 131).

b. Materials Recommended But Not Provided

(1) Laboratory balance
(2) Sample Grinder
(3) Distilled or deionized water
(4) Orbital/rotary shaker
(5) Micro-centrifuge tubes
(6) Graduated cylinder (150 – 200 mL)
(7) Mini-Pet pipette 100 µL (ACC 041)
(8) Sample cups with lids (ACC 012-50)
(9) Approved coffee filter (ACC 083)
(10) FREE Dilution Set – Disposables and Extraction Powder packets for 100 dilutions (ACC 034)
(11) QuickTox Dilution Set – Disposables for 100 dilutions when testing samples above the base range (ACC 080)
(12) 50 gram sample extraction set – additional Extraction Powder packets and sample extraction bags (ACC 035)

REVISION HISTORY

- Revision 0 (08/08/2016)

FLOW CHART

AQ 209 BG QuickTox Kit for QuickScan – Aflatoxin FREE for Corn