

# Test Kit Instructions

February 18, 2020

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## **ENVIROLOGIX TOTAL TOX FUMONISIN**

### **QUANTITATIVE FUMONISIN TEST KIT**

#### **FORWARD**

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 (TSD) by phone at 816-891-0417 or email at [Ajit.K.Ghosh@usda.gov](mailto:Ajit.K.Ghosh@usda.gov).

Refer to the Mycotoxin Handbook for information on use of this test kit in official FGIS 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 the Policies, Procedures, and Market Analysis Branch (PPMAB) by phone at 816-659-8403 or email at [Patrick.J.McCluskey@usda.gov](mailto:Patrick.J.McCluskey@usda.gov).

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## 1. GENERAL INFORMATION

The EnviroLogix Total Tox Fumonisin test kit uses lateral flow test strip technology that provides quantitative fumonisin test results.

Approved Test Kit Information	
<b>Test Kit Vendor:</b>	EnviroLogix Inc. 1-207-797-0300
<b>Test Kit Name:</b>	Total Tox Fumonisin
<b>Product Number:</b>	AQ 411 BG
<b>Effective Date of Instructions:</b>	02/18/2020
<b>Conformance Range:</b>	0.50 – 100 ppm
<b>Number of Analyses to Cover Conformance Range:</b>	2
<b>Type of Service:</b>	Quantitative
<b>Approved Commodities:</b>	Corn (including dent or field corn, corn meal, cracked corn, corn grits or polenta, and corn screenings)
<b>Extraction method:</b>	Corn: Add two EB17 dissolvable pouches and 150 mL of distilled or deionized water to a 50 g sample. Immediately shake vigorously for 10 seconds by hand. Then shake vigorously on a mechanical shaker (300 rpm) for 1 minute.
<b>Test Format:</b>	Lateral flow strip
<b>Detection Method:</b>	EnviroLogix QuickScan System, Software Version 5.1.1 update 1 or higher; EnviroLogix QuickScan II Reader, Software Version 5.1.1 update 1 or higher

## 2. PREPARATION OF TESTING MATERIALS AND EQUIPMENT

Bring all kit components, samples, and water used for extraction to room temperature (18 to 30°C) prior to use.

### a. Scanner Setup.

It is recommended that the QuickScan System and QuickScan II reader be calibrated daily and cleaned if necessary, after running the 'Clean Test'. Use the "Calibrate" button on the user-interface window to calibrate the scanner. Run a "Clean Test" with the "White Card" to verify if the reader surface is clean. If needed, clean the glass plate surface with screen cleaner using a lint-free, non-abrasive cloth. Run "Check Comb" to verify scanner optics. Detailed instructions for use of the reader systems are supplied with each unit, and can also be found at [www.envirologix.com/support/quickscan](http://www.envirologix.com/support/quickscan).

Ensure that the loaded QuickScan software is the appropriate version for the Total Tox Fumonisin test. QuickScan software version 5.1.1 update 1 or higher is required. Detailed instructions for upgrading can be found on the [EnviroLogix website](http://www.envirologix.com).

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. Additional MG barcodes for other non-approved commodities may be included, and if they are scanned, the QuickScan software will prompt users to select a Matrix Group (MG) when strips are scanned.

### b. Incubator Setup.

Turn on the incubator and set to 22°C for a minimum of 10 minutes before testing. Ensure the temperature display has stabilized and indicates 'OK' before starting the assay.

### c. Dilution Solution Preparation.

The Dilution Solution is used with the 10-100 ppm Quantitation Range (Dilution A Protocol). Prepare Dilution Solution by placing one EB17 pouch in an extraction container and add 150 mL of distilled or deionized water using a 250 mL graduated cylinder. Seal container and shake at least 1 minute or until pouch is completely dissolved. 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, so the pipetted portion will be turbid.

### 3. EXTRACTION PROCEDURES

a. Extraction of: Corn (field/dent corn, corn meal, cracked corn, corn grits/polenta, corn screenings)

- (1) Transfer 50.0 ± 0.2 grams ground sample to a hard-walled extraction container (ACC 099).
- (2) Add two dissolvable EB17 pouches to the extraction container.
- (3) Using a 250 mL graduated cylinder, add 150 mL of distilled or deionized water to the extraction container, seal the container securely and immediately shake vigorously by hand for 10 seconds to prevent the extraction powder from clumping and then shake the extraction mixture at high speed (300 rpm) on an orbital shaking platform for one minute.
- (4) Filter the extract by pouring onto an approved coffee filter (ACC 083) into a clean collection vessel and allow the sample to filter for 2 minutes. Then discard the filter paper and save the **Filtered Extract** for testing. **Filtered Extract** can be used for up to 3 hours.

### 4. SAMPLE PREPARATION FOR QUANTITATION

To avoid contamination, use a separate pipette tip for each transfer, and keep buffer and samples covered when not in use.

a. **For the 0.5 – 10 ppm quantitation range (Base Range Protocol):**

- (1) Using a 100 µL pipette, transfer 100 µL assay buffer DB5 into a clean Reaction Tube.
- (2) Using a 100 µL pipette, add 100 µL of **Filtered Extract** into the Reaction Tube and mix thoroughly by vortexing for 5 seconds or by pipetting up and down a minimum of 5 times with a 100 µL pipette tip.

- b. **For the 10 – 100 ppm quantitation range (Dilution A Protocol):**
- (1) If not previously prepared, make Dilution Solution as indicated in “Dilution Solution Preparation” section above.
  - (2) Using the variable 200-1000 µL pipette, transfer 700 µL of Dilution Solution into a blue dilution tube.
  - (3) Using a variable 20 -200 µL pipette, add 50 µL of **Filtered Extract** into the dilution tube from step (2) above and mix thoroughly by vortexing for 5 seconds or by pipetting up and down a minimum of 5 times with a 1000 µL pipette tip. This is **Diluted Extract A** and can be used for up to 3 hours.
  - (4) Using a 100 µL pipette, transfer 100 µL of assay buffer DB5 into a Reaction Tube.
  - (5) Using a 100 µL pipette, add 100 µL **Diluted Extract A** ((from step (3)) into the Reaction Tube and mix thoroughly by vortexing for 5 seconds or by pipetting up and down a minimum of 5 times with the 100 µL pipette tip.

## 5. TEST PROCEDURES

- a. Place the Reaction Tube into the incubator and allow to equilibrate for 2 minutes at 22°C.
- b. Place a test strip into the reaction tube and develop the strip for 4 minutes. The arrow tape end of the strip should point into the reaction tube.
- c. Immediately after completion of the development time, cut off and discard the bottom of the strip covered by the arrow tape.
- d. Insert test strip into the QuickScan reader for quantitation.

**For QuickScan System:** Place the test strip face down in the carrier with the barcoded end closest to the handle.

**For QuickScan II Reader:** Place the test strip face up in the strip tray with the barcoded end closest to the operator (see diagram on the strip tray).

- e. Insert the carrier into the reader and touch/click on the “Read Test” area of the computer screen.

- f. From the pop-up menu prompt, choose the appropriate matrix group as follows:

Matrix Group ID	Sample Matrices
FM MG1	Corn (including Field/Dent Corn, Corn Meal, Cracked Corn, Corn Grits/Polenta, Corn Screenings)

- g. The Results screen will open when the test is complete.
- h. On the Results screen, the software defaults to 1:1 under the Dilution tab. No adjustment is required for samples that were run in the 0.5 – 10 ppm quantitation range. For samples that originally read >10 ppm and are being re-tested in the Dilution A Protocol, use the dilution column pull-down menu to select 1:A.
- i. Add sample information and comments to the results screen, then close the window. Closing this screen will save the results automatically in the datalog file.
- j. A final result less than 7.4 ppm (using 10 – 100 ppm quantitation range) is indicative of a problem, and troubleshooting is needed. Verify the procedure is being followed properly. Perform the analysis using 0.5 – 10 ppm quantitation range and only perform the analysis using 10 – 100 ppm quantitation range again if the value is greater than 10 ppm.

## 6. 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@usda.gov](mailto:Patrick.J.McCluskey@usda.gov)).

## 7. STORAGE CONDITIONS AND PRECAUTIONS

- a. Storage Conditions.
- (1) Test kits should be stored refrigerated between 2 to 8°C; prolonged exposure to high temperatures may adversely affect test results.
  - (2) Bring kit components and water for extraction to ambient temperature (18 to 30°C) before use. Do not open the desiccated canister until ready to use the strips.

b. Precautions and Testing Notes.

- (1) Developing the test for the specified time and reading the test strip promptly is required for accurate results.
- (2) Do not use the test kits beyond the noted expiration date.
- (3) Protect all components from hot or cold temperatures, when not in use. Do not leave in direct sunlight or in a vehicle.
- (4) Follow the FGIS-issued procedures to run the test. Deviation from this protocol may invalidate test results reported using the test kit.
- (5) Take care not to contaminate DB5 Buffer solution. Use a new pipette tip for each measurement and keep closed when not in use.
- (6) Avoid foam and particulates during pipetting and ensure that the pipette tip does not become clogged with particulates.
- (7) Proper mixing and wetting of the sample along with accurate pipetting are essential for accurate results. To prevent the extraction powder from clumping during extraction with EB17, sample must be shaken immediately after water addition in a hard-walled vessel.
- (8) Ensure your QuickScan System/QuickScan II Reader has been updated with the most recent software version 5.1.1 update 1 or later.

**8. EQUIPMENT AND SUPPLIES**

(Catalog No. shown in parentheses)

a. Materials Supplied in Test Kits.

- (1) 50 QuickTox Strips packed in a moisture-resistant canister
- (2) 50 dissolvable EB17 pouches
- (3) 50 Reaction Tubes
- (4) 100 pipette tips (1–200 µL)
- (5) DB5 Buffer
- (6) Multi-Matrix Barcode Card, kit lot specific



b. Materials Required but not Provided.

- (1) QuickScan System\* (ACC 131) of QuickScan II Reader (ACC 331)
- (2) Incubator unit\* (ACC BSH301-12458)
- (3) 50g Sample Extraction Set\*: Additional Extraction Pouches and Sample Cups (ACC 099)
- (4) 250 mL Class A graduated cylinder (Fisher Scientific, 03-007-42)
- (5) Approved Coffee filters\* (ACC 083)
- (6) Filtering cups (5 oz.)\* (ACC 012 50)
- (7) FGIS-approved type scale with a minimum division size of 0.1 gram
- (8) 100 µL fixed-volume pipette\* (ACC 041)
- (9) 100-1000 µL variable volume pipette \* (ACC 1303-Pro-1000)
- (10) 100-1000 µL pipette tips \*(20-0127)
- (11) 20-200 µL variable volume pipette (Fisher Scientific FBE00200 or other vendors)
- (12) Blue Dilution Tubes \*(ACC 103)
- (13) Bunn grinder or equivalent (see FGIS Mycotoxin Handbook)
- (14) 20-mesh screen (available through Seedburo or other vendors)
- (15) Orbital/rotary shaker (VWR Cat#89032-096, Model#3500)
- (16) Vortex mixer (Fisher's catalog, catalog # 02-215-414)
- (17) Timer
- (18) Scissors
- (19) Distilled or deionized water

\*Available as Accessories

**9. REVISION HISTORY**

Effective 02/18/2020

## 10. FLOW CHART

### AQ 411 BG Total Tox Fumonisin – Corn

#### **Set-up:**

1. Equilibrate all reagents, including samples, strips, buffer and water, at room temperature before use
2. Turn on incubator to 22°C for at least 10 minutes before use
3. For each QuickScan, calibrate, run 'Clean Test', and run Check Comb
4. Scan the MMBC into the QuickScan System and QuickScan II Reader
5. Place coffee filters in filtering cups
6. Make Dilution Solution (for diluting high positives):  
1 dissolvable EB17 + 150 mL diH<sub>2</sub>O

#### **Extraction:**

1. 50g ground corn + 2 dissolvable EB17 + 150 mL diH<sub>2</sub>O in hard-walled cup
2. Immediately shake 10 sec by hand, then shake 1 min on orbital shaker (300 rpm)
3. Pour through coffee filter; wait 2 minutes
4. Use filtrate beneath filter for testing

#### **Base Range (0.5 – 10 ppm)**

1. In a reaction tube, add 100 µL DB5 buffer + 100µL filtered extract; mix well
2. Place in 22°C incubator 2 min
3. Add strip and develop for 4 min
4. Cut-off bottom pads and immediately read on either a QSS System or a QSS II Reader
5. Select MG1 for corn
6. Select 1:1 in the dilution tab on results screen
7. Record sample notations

If results are >10 ppm, repeat testing using Dilution A protocol.

#### **Dilution A Range (10 – 100 ppm)**

- A1. In a separate tube, add 700 µL Dilution Sol'n + 50 µL filtered extract; mix well
- A2. In a reaction tube, add 100 µL DB5 buffer + 100 µL diluted extract from A1 above; mix well
- A3. Place in 22°C incubator 2 min
- A4. Add strip and develop for 4 min
- A5. Cut-off bottom pads and read immediately on either a QSS System or a QSS II Reader
- A6. Select MG1 for corn
- A7. Select 1:A in the dilution tab on results screen
- A8. Record sample notations