

Test Kit Instructions

November 5, 2020

ENVIROLOGIX TotalTox Zearalenone

QUANTITATIVE ZEARALENONE 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.

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.

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1. GENERAL INFORMATION

The EnviroLogix TotalTox Zearalenone test kit uses lateral flow test strip technology that provides quantitative zearalenone test results.

| Approved Test Kit Information | |
|---|---|
| Test Kit Vendor: | EnviroLogix Inc. 1-207-797-0300 |
| Test Kit Name: | TotalTox Zearalenone |
| Product Number: | AQ 412 BG |
| Effective Date of Instructions: | 11/5/2020 |
| Conformance Range: | 100 – 1000 ppb |
| Number of Analyses to Cover Conformance Range: | 2 |
| Type of Service: | Quantitative |
| Approved Commodities: | Corn (including dent or field corn, corn meal, cracked corn, corn grits or polenta, and corn screenings only), wheat (including wheat screenings only) |
| Extraction method: | 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. |
| Test Format: | Lateral flow strip |
| Detection Method: | EnviroLogix QuickScan System, Software Version 5.1.1 update 2 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 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.

Ensure that the loaded QuickScan software is the appropriate version for the Total Tox Zearalenone test. QuickScan software version 5.1.1 update 2 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 corn 500-1000 ppb 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 only) and wheat (wheat screenings only). The sample to be tested should be collected and prepared according to accepted sampling techniques (see **Mycotoxin Handbook**).
- (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) **Corn (including field/dent corn, corn meal, cracked corn, corn grits/polenta, corn screenings only):** 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.
 - (5) **Wheat (wheat screenings):** Transfer the extract to a microcentrifuge tube. Centrifuge the extract for 30 seconds at 2000 x g. Transfer the clear top layer (supernatant) to a clean vessel for testing. The **Centrifuged 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. **Corn (including field/dent corn, corn meal, cracked corn, corn grits/polenta, corn screenings only): For the 100 – 500 ppb quantitation range (Base Range Protocol):**

- (1) Using a 100 μ L pipette, transfer 100 μ L assay buffer DB5 into a clean Reaction Tube.
 - (2) Using a 100 μ L pipette, add 100 μ L 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 the 100 μ L pipette.
- b. **Corn (including field/dent corn, corn meal, cracked corn, corn grits/polenta, corn screenings only): For the 500 – 1000 ppb quantitation range (Dilution A Protocol):**
- (1) If not previously prepared, make the Dilution Solution as indicated in “Dilution Solution Preparation” section above.
 - (2) Using the variable 100-1000 μ L pipette, transfer 800 μ L of Dilution Solution into a dilution tube.
 - (3) Using a 100 μ L pipette, add 100 μ L 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 μ L pipette and tip. This is **Diluted Extract A** and can be used for up to 3 hours.
 - (4) Using a 100 μ L pipette, transfer 100 μ L of assay buffer DB5 into a Reaction Tube.
 - (5) Using a 100 μ L pipette, add 100 μ L **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 μ L pipette.
- c. **Wheat (wheat screenings only): For the 100 – 1000 ppb quantitation range (Base Range Protocol)**

- (1) Using a 20-200 µL variable pipette, add 200 µL assay buffer DB5 into a clean Reaction Tube.
- (2) Using a 100 µL pipette, add 100 µL of **Centrifuged 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.

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.
- 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 |
|-----------------|--|
| ZN MG1 | Corn (including Field/Dent Corn, Corn Meal, Cracked Corn, Corn Grits/Polenta, Corn Screenings) |
| ZN MG2 | Wheat 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 corn: 100 – 500 ppb or Wheat: 100-1000 ppb quantitation range. For corn samples that originally read >500 ppb 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 300 ppb (using 500 – 1000 ppb quantitation range) is indicative of a problem, and troubleshooting is needed. Verify the procedure is being followed properly. Perform the analysis using the 100 – 500 ppb quantitation range and only perform the analysis using 500 – 1000 ppb quantitation range again if the value is greater than 500 ppb.

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).

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 has been updated with the most recent software version 5.1.1 update 2 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 Reaction Tubes
- (3) 100 pipette tips (1–200 μ L)
- (4) DB5 Buffer
- (5) Multi-Matrix Barcode Card, kit lot specific

b. Materials Required but not Provided.

- (1) QuickScan System* (ACC 131)
- (2) EB17 Extraction Buffer pouches (ACC 117)
- (3) Incubator unit* (ACC BSH301-12458)
- (4) 50g Sample Extraction Set*: Additional Extraction Pouches and Sample Cups (ACC 099)
- (5) 250 mL Class A graduated cylinder (Fisher Scientific, 03-007-42)
- (6) Approved Coffee filters* (ACC 083)
- (7) Filtering cups (5 oz.)* (ACC 012 50)
- (8) Scale capable of measuring 50 grams – see Mycotoxin Handbook
- (9) 100 μ L fixed-volume pipette* (ACC 041)
- (10) 100-1000 μ L variable volume pipette * (ACC 1303-Pro-1000)

- (11) 100-1000 µL pipette tips *(ACC 20-0127)
- (12) 20-200 µL variable volume pipette (Fisher Scientific FBE00200 or other vendors)
- (13) Blue Dilution Tubes *(ACC 103)
- (14) Bunn grinder or equivalent (see FGIS Mycotoxin Handbook)
- (15) 20-mesh screen (available through Seedburo or other vendors)
- (16) Orbital/rotary shaker (VWR Cat#89032-096, Model#3500)
- (17) Vortex mixer (Fisher catalog # 02-215-414)
- (18) Timer
- (19) Scissors
- (20) Distilled or deionized water
- (21) *Additional DB5 buffer **for wheat** (KR-266-7).
- (22) *Centrifugation Set **for wheat** (ACC 010)
- (23) *Microcentrifuge **for wheat** (ACC 064 E)

*Available as Accessories

9. REVISION HISTORY

Effective 11/5/2020

10. FLOW CHARTS

AQ 412 BG TotalTox Zearalenone – 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. QuickScan Reader: Calibrate, run 'Clean Test', and run Check Comb
4. Scan the MMBC into the QuickScan System
5. Place coffee filters in filtering cups
6. Make Dilution Solution (for diluting high positives):
1 dissolvable EB17 + 150 mL diH₂O

Extraction:

1. 50g ground corn + 2 dissolvable EB17 pouches + 150 mL diH₂O 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 (100 – 500 ppb)

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 QuickScan System
5. Select MG1 for corn
6. Select 1:1 in the dilution tab on results screen (this is the software default)
7. Record sample notations

If results are >500 ppb, repeat testing using Dilution A protocol.

Dilution A Range (500 – 1000 ppb)

- A1. In a separate tube, add 800 µL Dilution Sol'n + 100 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 QuickScan System
- A6. Select MG1 for corn
- A7. Select 1: A in the dilution tab on results screen
- A8. Record sample notations

AQ 412 BG TotalTox Zearalenone – Wheat

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. QuickScan Reader: Calibrate, run 'Clean Test', and run Check Comb
4. Scan the MMBC into the QuickScan System
5. Make Dilution Solution (for diluting high positives):
1 dissolvable EB17 + 150 mL diH₂O

Extraction:

1. 50g ground corn + 2 dissolvable EB17 pouches + 150 mL diH₂O in hard-walled cup
2. Immediately shake 10 sec by hand, then shake 1 min on orbital shaker (300 rpm)
3. Transfer extract to a microcentrifuge tube
4. Centrifuge for 30 seconds at 2000 x g
5. Transfer the clear top layer (supernatant) to a clean vessel
6. Use the supernatant for testing

Base Range (100 – 1000 ppb)

1. In a reaction tube, add 200 µL DB5 buffer + 100µL clarified 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 QuickScan System
5. Select MG2 for Wheat
6. Select 1:1 in the dilution tab on results screen (this is the software default)
7. Record sample notations

If results are >1000 ppb, report the results as '>1000 ppb'.