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 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@usda.gov.
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1. GENERAL INFORMATION

The EnviroLogix QuickTox Kit for QuickScan DON Flex test kit uses lateral flow test strip technology to provide quantitative DON (vomitoxin) test results.

<table>
<thead>
<tr>
<th>Approved Test Kit Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Kit Vendor:</td>
</tr>
<tr>
<td>EnviroLogix Inc. 1- 207-797-0300</td>
</tr>
<tr>
<td>Test Kit Name:</td>
</tr>
<tr>
<td>QuickTox Kit for QuickScan DON FLEX</td>
</tr>
<tr>
<td>Product Number:</td>
</tr>
<tr>
<td>AQ 304 BG</td>
</tr>
<tr>
<td>Effective Date of Instructions:</td>
</tr>
<tr>
<td>Instructions Revision Number:</td>
</tr>
<tr>
<td>Conformance Range:</td>
</tr>
<tr>
<td>0.50 – 30 ppm</td>
</tr>
<tr>
<td>Number of Analyses to Cover Conformance Range:</td>
</tr>
<tr>
<td>Type of Service:</td>
</tr>
<tr>
<td>Quantitative</td>
</tr>
<tr>
<td>Approved Commodities:</td>
</tr>
<tr>
<td>corn, (field/dent corn, corn meal, corn flour, cracked corn, corn grits/polenta, corn screenings) wheat (whole grain wheat flour, wheat middlings, wheat red dog, wheat flour 2nd clear, wheat screenings), distillers dried grains with solubles (DDGS), wheat bran, barley, malted barley, corn germ, corn gluten feed (CGF), corn gluten meal.</td>
</tr>
<tr>
<td>Extraction method:</td>
</tr>
<tr>
<td>Add 250 mL of distilled, or deionized water to a 50 g sample. Fully wet the sample then shake vigorously on a mechanical shaker (300 rpm) for 30 seconds.</td>
</tr>
<tr>
<td>Test Format:</td>
</tr>
<tr>
<td>Lateral Flow Strip</td>
</tr>
<tr>
<td>Detection Method:</td>
</tr>
<tr>
<td>EnviroLogix QuickScan System, Software Version 4.9.4, Update 4</td>
</tr>
</tbody>
</table>
2. PREPARATION OF TESTING MATERIALS AND EQUIPMENT

Allow all samples, water used for extraction, and test kit materials to equilibrate to room temperature before use.

a. **Scanner Setup.**

   It is recommended that the QuickScan System be cleaned and calibrated daily. Clean the glass plate surface with screen cleaner using a lint-free, non-abrasive cloth. Use the “Calibrate” button on the user-interface window to calibrate the scanner. Run a “Clean Test” with the “White Card” to verify your glass cleaning process. Run “Check Comb” to verify scanner optics. Detailed instructions for use of the QuickScan System 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 DON-Flex test. QuickScan software version 4.9.4 update 2 or later is required. Detailed instructions for upgrading can be found at www.envirologix.com/quickscan.

   Scan the Multi-Matrix Barcode Card (MMBC) provided with each kit prior to using a new lot of test strips. Scanning the MMBC is only required once per kit lot. If only testing matrices that are included in the DF MG1 Group, fold the Multi Matrix Barcode and scan only the DF MG1 barcode. If only testing matrices that are included in the DF MG2 Group, fold and scan the DF MG2 barcode. This allows the software to skip the step which prompts users to select a Matrix Group.

b. **Incubator Setup.**

   Turn on the incubator and set to 22°C at least 10 minutes before testing. Ensure the temperature display has stabilized and indicates ‘OK’ before starting the assay.

3. EXTRACTION PROCEDURES

a. **Transfer 50.0 ± 0.2 grams ground sample to an extraction bag.**

b. **Add 250 mL of distilled, or deionized water and seal the bag. Ensure the sample is completely wetted.**

c. **Vigorously shake the bag on an orbital shaker at 300 rpm for 30 seconds.**

d. **Filter the sample through an approved coffee filter (EnviroLogix ACC 083, Part No. 11434) into a clean collection vessel (not provided) and allow sample extract to filter for 2 minutes (use timer).**

e. **Discard the filter paper and save the filtered sample extract for testing. Filtered sample extract can be used for up to 3 hours when stored in a clean and closed container.**
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 – 8.0 ppm quantitation range:

(1) Transfer 100 microliters (µL) of lot-specific DB6 buffer solution into a clean reaction tube. Take care not to contaminate DB6 buffer-use a new tip for each test and keep covered when not in use.

(2) Using another new pipette tip, add 100 µL of the filtered extract into the reaction tube with the DB6 buffer, and thoroughly mix by pipetting up and down at least 5 times.

b. For the 8 – 30 ppm quantitation range:

(1) Using 100 µL pipettor, transfer 100 µL of the filtered extract into a dilution vessel (not provided, see accessories).

(2) Using 1000 µL pipettor, add 700 µL of distilled, or deionized water into the dilution vessel containing 100 µL of the filtered extract and mix well by pipette up and down at least 5 times. This is diluted extract.

(3) Using a new pipette tip, transfer 100 µL of lot-specific DB6 buffer solution into a clean reaction tube.

(4) Using another new pipette tip, add 100 µL of the diluted extract into the reaction tube containing DB6 buffer solution and thoroughly mix by pipetting up and down at least 5 times.

5. TEST PROCEDURES

a. Place the reaction tube in the incubator and equilibrate for 2 minutes at 22°C

b. Place a test strip into the reaction tube, and develop the strip for two minutes. The arrow tape end of the strip should point into the reaction tube.

c. Immediately after completion of the 2-minute development, cut off and discard the bottom of the strip covered by the arrow tape.

d. Insert test strip into the QuickScan reader for quantitation. Place the test strip face down in the carrier with the barcoded end closest to the handle.

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:

<table>
<thead>
<tr>
<th>Matrix Group ID</th>
<th>Samples Matrices</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF MG1</td>
<td>Wheat</td>
</tr>
<tr>
<td>DF MG2</td>
<td>Corn, Field/Dent Corn, Corn Meal, Corn Flour, Cracked Corn, Corn Grits/Polenta, Corn Screenings</td>
</tr>
<tr>
<td>DF MG3</td>
<td>Wheat Flour, Wheat Flour 2nd Clear, Wheat Screenings</td>
</tr>
<tr>
<td>DF MG4</td>
<td>White Wheat Flour, Wheat Bran</td>
</tr>
<tr>
<td>DF MG5</td>
<td>Wheat Middlings</td>
</tr>
<tr>
<td>DF MG6</td>
<td>Wheat Red Dog</td>
</tr>
<tr>
<td>DF MG7</td>
<td>Distillers Dried Grains w/ Solubles (DDGS)</td>
</tr>
</tbody>
</table>

The results screen will open when the test is complete.

g. 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 0.5-8.0 ppm quantitation range. For samples that originally read >8.0 ppm and are being re-tested following the 8.0-30 ppm quantitation range, use the Dilution column pull-down menu to select 1:A.

h. 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.

A final result (using 8 – 30 ppm quantitation range) less than 5.6 ppm is indicative of a problem, and troubleshooting is needed. Verify the procedure is being followed properly. Perform the analysis using 0.5 – 8 ppm quantitation range and only perform the analysis using 8 – 30 ppm quantitation range again if the value is greater than 8 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@udsa.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 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 full two minutes and reading the test strip promptly are 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. Do not leave in direct sunlight or in a vehicle.

(4) Follow the GIPSA-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 DB6 buffer solution--use a new pipette tip for each measurement, and keep closed when not in use. Samples that are not thoroughly mixed and/or accurately pipetted will adversely affect test results.

(6) Avoid foam and particulates during pipetting, and ensure that the pipette tip does not become clogged with particulates.

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

(8) Ensure your QuickScan System has been updated with the most recent software version.

(9) The DB6 buffer is lot specific, do not use buffer from another lot.
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
   (4) DB6 Buffer, kit lot specific
   (5) Multi-Matrix Barcode Card, kit lot specific

b. Materials Required but not Provided:
   (1) QuickScan System* (ACC 131)
   (2) Incubator base* (ACC BSH300)
   (3) Incubator block* (ACC BSH1000-1213)
   (4) Vessels to hold 800 µL for dilution of samples * (ACC 098)
   (5) Approved Coffee Filters* (ACC 083)
   (6) Graduated cylinder (100 mL)* (ACC 068)
   (7) Pipettes
       - to deliver 100-µL* (ACC 041)
       - to deliver 700-µL* (ACC 1303)
   (8) Extraction bags * (ACC 066)
   (9) Filtering cup (5 oz.) from Evergreen Scientific (cat#300562502KM)
   (10) Scale for weighing samples (available through Fisher Scientific)
   (11) BUNN grinder or equivalent (available through BUNN or other vendors)
   (12) 20-mesh screen (available through Seedburo or other vendors)
   (13) Orbital/rotary shaker (VWR Cat#89032-096, Model#3500)
   (14) Timer
   (15) Scissors
   (16) Distilled, or deionized water

*Available as Accessories from EnviroLogix

9. REVISION HISTORY

Revision 1 (1/26/2017)

• Barley, malted barley, corn germ, corn gluten feed (CGF), corn gluten meal were approved as additional commodities and the test procedure was updated.

Revision 0 (09/28/2016)
10. FLOW CHART

QuickTox Kit for QuickScan DON Flex (AQ 304 BG)

**EXTRACTION**

Grind

50 g

250 mL Water

Shake 30 seconds

Filter (coffee filter) for 2 minutes

**ASSAY RESULTS**

0.5 – 8 ppm

100 µL DB6

100 µL filtered extract

Reaction Tube, mix

Place tube @ 22°C in incubator, for 2 min.

Add assay strip to tube (in incubator) 2 min.

Cut off bottom of the strip

Read in QuickScan System

Select sample-appropriate Matrix Group, maintaining default Dilution 1:1

Record sample information

If Results >8.0 ppm, retest

8 – 30 ppm

700 µL water

100 µL filtered extract

Mix in additional vessel

100 µL DB6

100 µL

Reaction Tube, mix

Place tube @ 22°C in incubator, for 2 min.

Add assay strip to tube (in incubator) 2 min.

Cut off bottom of the strip

Read in QuickScan System

Select sample-appropriate Matrix Group & Dilution 1:A

Record sample information