

Test Kit Instructions

September 29, 2020

PerkinElmer: AuroFlow AQ DON Strip Test **Using QuickStar Horizon Strip Reader**

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GENERAL INFORMATION

The AuroFlow AQ DON Strip Test is a quantitative rapid lateral flow assay (strip test) kit designed to quantitatively determine the amount of deoxynivalenol (DON) residues in wheat and corn. This kit utilizes an environmentally-friendly aqueous extraction method. Deoxynivalenol present in the sample extraction interacts with the strip (device) components and influences color intensity on the test and control lines. Developed test strips are inserted into an electronic strip reader, which reports deoxynivalenol contamination of the sample in parts per million (ppm). This test is designed for rapid field or reference laboratory settings.

Please read all instructions thoroughly prior to performing the test.


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 the use of this test kit for 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 (816-659-8403 or Patrick.J.McCluskey@usda.gov).

Approved Test Kit Information

Kit Test Vendor:	PerkinElmer Inc. (1-512-707-8993)
Test Kit Name:	AuroFlow AQ DON Strip Test
Product Number:	FOOD-1414-01
Effective Date of Instructions:	9/29/2020
Conformance Range:	0.50 - 30 ppm
Number of Analyses to Cover Conformance Range:	2
Type of Service:	Quantitative
Approved Commodities:	Corn (field/dent corn, corn meal, cracked corn, corn grits/polenta, corn screenings), wheat (whole grain wheat flour, wheat middlings, wheat red dog, wheat flour 2 nd clear, and wheat screenings).
Extraction Method:	Shake 50 grams of sample with 200 mL AQ Extraction Buffer for 1 minute and 30 seconds.
Test Format:	Lateral Flow Strip
Detection Method:	QuickStar Horizon Strip Reader (Catalog # FOOD-6006-01)

PREPARATION OF TESTING MATERIALS AND EQUIPMENT

1. Equilibration of Kit Contents
 - a. Remove the kit and all its accompanying contents from 4 °C storage. Allow the kit to rest for 1 hour at room temperature (18 – 30 °C) before performing any determinations.
 - b. Do not use the kit outside an 18 – 30 °C (65 – 86 °F) temperature range.
2. Preparation of AQ Extraction Buffer:
 - a. AQ Extraction Buffer must be prepared prior to sample extraction.
 - b. To prepare the AQ Extraction Buffer, add 550 mL of distilled or deionized water using a 1 L graduated cylinder and 300 µL of AQ Add-In Solution using a 1000 µL pipettor into a clean 1 L bottle.
 - c. Transfer the entire contents of one (1) Large AQ Powder Pack (Catalog # FOOD-4005-200) into the bottle with a wide-mouth funnel and tap to dislodge solids. Manually shake for 1 minute to dissolve completely. Label it with the date and the analyst's name. Store at room temperature for up to 3 months.
 1. Alternatively: Add a magnetic stir bar and stir at high speed with a magnetic stir plate.
3. Preparation of Equipment
 - a. Turn on orbital shaker and confirm calibration status.
 - b. Plug the reader and printer into their respective power supplies. Power on the reader. Connect the printer to the reader via a USB cord.
 - c. Calibrate the strip reader before performing any extractions:
 1. QuickStar Horizon Strip Reader
 - a. Power on the reader.
 - b. Select user or enter user information.
 - c. Identify the two QR Calibration Codes matching the kit lot #.
 - d. From the main menu, navigate to settings via the gear icon .
 - e. Select "Lot ID".
 - f. Scan each supplied QR code. The lot-specific calibration information will automatically be saved to the matching Lot ID #.

SAMPLE PREPARATION AND EXTRACTION PROCEDURES

1. Extraction procedure for corn (field/dent corn, corn meal, cracked corn, corn grits/polenta, corn screenings), wheat (whole grain wheat flour, wheat middlings, wheat red dog, wheat flour 2nd clear, and wheat screenings). The sample to be tested should be collected and prepared according to accepted sampling techniques (see **Mycotoxin Handbook**).
 - a. Transfer 50 ± 0.2 grams of ground sample into a Large Extraction Bag (Catalog # FOOD-4009-200)
 - b. Transfer 200 mL of AQ Extraction Buffer into container using a 250 mL graduated cylinder.
 - c. Seal the Large Extraction Bag and briefly shake to moisten the sample and break the lump.
 1. Shake the sample at 250 rpm with an orbital shaker for 1 minute and 30 seconds.
 2. To clarify the extract, prepare the filtration unit: Fold one filter (Catalog # FOOD-4010-01) and place it inside of a filter funnel. Then place the funnel into a clean conical tube.
 3. Using a serological or transfer pipet, transfer approximately 10 mL of liquid extract into the assembled filter funnel. Avoid transferring solids or foam.
 4. Allow the sample to filter by gravity until approximately 2 – 3 mL of filtrate has been collected (approximately 2 minutes for corn and 7 minutes for wheat). This filtrate is the “**Sample Extract**”.
 5. Remove the funnel and filter from the conical tube and set aside. Residual solids and waste on the filter can be discarded.
 6. Cap the conical tube, and then briefly vortex the contents to mix.
 7. The sample extract must be tested within 1 hour, as described below.
 8. Follow local and organizational guidance for disposing of deoxynivalenol-contaminated samples.

TESTING PROCEDURE

1. Reader Setup
 - a. Select the appropriate program on the QuickStar Horizon Strip Reader and select the correct lot ID #. Enter any desired sample information.

1. For the “Default” quantitation range (0.5 – 5 ppm):
 - a. Select the AuroFlow “**AQ DON Low**” program
 2. For the “Highly Contaminated” quantitation range (5 – 30 ppm):
 - a. Select the AuroFlow “**AQ DON High**” program
 3. Identify the commodity type when prompted for the ‘Dual Curve’.
 - a. For testing corn samples, select ‘**Curve #1**’ when prompted.
 - b. For testing wheat samples, select ‘**Curve #2**’ when prompted.
2. Preparation of **Sample Mix** and **Diluted Sample Mix**
- a. **“Default” Quantitation Range (0.5 – 5 ppm):** All samples must be initially tested using the following procedure:
 1. Using the 1000 µL pipettor, transfer 2.6 mL of Running Buffer to a Transport Tube: Complete this addition by pipetting in the following increments: 1 mL + 1 mL + 600 µL with a fresh new tip for each addition.
 2. Using the 200 µL pipettor, transfer 100 µL of the Sample Extract into the same tube containing Running Buffer. Pipet up and down 3 times, and then vortex on high for 5 seconds to mix. This is the “**Sample Mix**”.
 3. Test the **Sample Mix** within 30 minutes, as described in “Sample Analysis”.
 - b. **“Highly Contaminated” Quantitation Range (5.0 – 30 ppm):** If the reader outputs a result greater than 6 ppm, re-evaluate the sample using the following dilution procedure:
 1. Using the 1000 µL pipettor, transfer 700 µL of High Dilution Buffer into a new Capped Tube.
 2. Using the 200 µL pipettor, transfer 100 µL of the **Sample Extract** into the above tube. Pipet up and down 3 times, and then vortex on high for 5 seconds to mix. This is the “**Diluted Sample Extract**”.
 3. Using the 1000 µL pipettor, transfer 2.6 mL of Running Buffer to a Transport Tube: Complete this addition by pipetting in the following increments: 1 mL + 1 mL + 600 µL with a fresh new tip for each addition.
 4. Using the 200 µL pipettor, transfer 100 µL of the Diluted Sample Extract to the same tube with Running Buffer. Pipet up and down 3 times, and then vortex on high for 2 seconds to mix. This is the “**Diluted Sample Mix**”.
 5. Test the **Diluted Sample Mix** within 30 minutes, as described in ‘Sample Analysis’.

3. Sample Analysis

- Using the 200 μL pipettor, transfer 150 μL of the **Sample Mix** (Quantitation range: 0.5 – 5 ppm) or **Diluted Sample Mix** (Quantitation range: 5.0 – 30 ppm) to an appropriate Capless Tube in a tube rack at room temperature.
- Add a new test strip to the Capless Tube with the arrows pointing down and allow the strip to develop for 4 minutes.
- Remove the strip from the Capless Tube immediately at 4 minutes. Gently touch the end of the test strip onto an absorbent paper towel or Kimwipe.
- Visually inspect strips immediately (**Figure 1**), as described below. Valid strips must be analyzed within 1 minute of removal. Do not read invalid strips.

4. Visual Inspection

- The test strip is INVALID if any of the following are observed:
 - Test (T) or Control (C) line is uneven or “broken”.
 - Control (C) line is absent.
 - Gold (pink conjugate) does not flow past T and C lines.

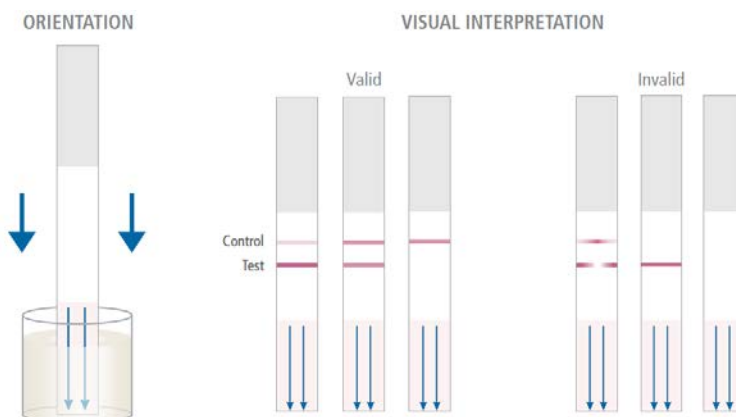






Figure 1. Test Strip Orientation and Test Interpretation

- Do not read INVALID test strips in reader.
- If test strip is invalid, prepare fresh **Sample Mix** or **Diluted Sample Mix** (as outlined in the ‘Sample Mix Preparation’ section of the Testing Procedure) and re-test with a new strip.
- If test strip is valid (no observed defects and even line development), proceed to “Interpretation” below to read strip.

5. Interpretation

- Only use readers calibrated with lot-specific QR codes matching the strip lot.

- b. Ensure that the correct Lot ID # and corresponding sample type was selected for the determination.
 - 1. “-1” after the Lot ID # indicates that the option ‘Curve #1’ was selected (for corn samples).
 - 2. “-2” after the Lot ID # indicates that the option ‘Curve #2’ was selected (for wheat samples).
- c. QuickStar Horizon Strip Reader
 - 1. Insert a valid strip into the cassette, with arrows inserted first, and with “#1” facing up Select “Run Test”. Select correct Lot ID #.
 - 2. If desired, enter sample ID.
 - 3. The reader should automatically record a result.
 - a. If the reader does not automatically read the strip, select the “” icon to read the strip manually.
 - 4. Verify that the reader displays both control and test lines in the window, otherwise the test is invalid. The strip may be re-read if necessary using the “” icon.
 - a. Invalid results require repeating the analysis from the “Sample Mix Preparation” step.
 - 5. Print the result by pressing the printer icon “”.
- d. If the reader reports a value over 6 ppm, follow the testing procedure outlined for “Highly Contaminated” Quantitation Range (5 – 30 ppm).
- e. If the QuickStar Horizon Strip Reader reports an “Invalid” result, verify that the test strip is properly inserted into the cassette and reader. If the reader again reports an “Invalid” result, perform a calibration verification as follows:
 - 1. In the top left Settings “” menu select “Diagnostic Check”.
 - 2. Following the on-screen instructions: insert the white crosshair cartridge into the cassette slot, rounded end down. Make sure the cartridge is firmly seated all the way at the bottom of the slot.
 - 3. Press “Start” and allow the reader to complete the diagnostic.
 - 4. Confirm that the date of the last diagnostic has changed to the current date and the status is displayed as “Valid”.
 - 5. If the reader continues to report “Invalid” results, contact Bioo.Support@PerkinElmer.com for support.

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).

STORAGE CONDITIONS AND PRECAUTIONS

1. Storage Conditions:

- a. Store the test strips refrigerated at 4 °C in the closed original container.
- b. Store the Running Buffer and High Dilution Buffer bottles refrigerated at 4 °C.
- c. Store Large AQ Powder Packs and AQ Add-in Solution with the kit at 4 °C until preparation of AQ Extraction Buffer.
- d. Store prepared AQ Extraction Buffer at room temperature (18 – 30 °C) for up to 3 months. Discard after 3 months or if the solution becomes turbid before 3 months.

2. Precautions:

- a. Do not leave test strip canister open for prolonged periods of time; remove strips as needed and re-close the canister.
- b. Do not perform determinations without equilibrating the test kit to room temperature.
- c. Do not perform more than 4 determinations simultaneously.
- d. Only use Running Buffer and High Dilution Buffer with test strips from the matching kit lot.
- e. AQ Extraction Buffer (not lot specific) is non-hazardous and may be disposed as normal waste.
- f. Do not open the Large AQ Powder Packs until ready to use.
- g. Do not use test kits or components beyond the expiration date.
- h. The QuickStar Horizon Strip Reader cassette may accumulate residue after extended use, in which case it may be wiped clean with distilled water applied to a Kimwipe. This residue build-up may be reduced by gently touching the freshly-developed strip on a paper towel or Kimwipe before inserting into the cassette.

EQUIPMENT AND SUPPLIES

1. Required materials provided in the AuroFlow AQ DON Strip Test (Catalog # FOOD-1414-01) kit:
 - a. 25 Test Strips
 - b. 3 bottles of Running Buffer
 - c. 1 bottle of High Dilution Buffer
 - d. 25 Filters
 - e. 25 Capless Tubes
 - f. 13 Capped Tubes
 - g. 25 Transfer Pipets, 3 mL
 - h. 2 Calibration Codes (lot-specific)
2. Required materials provided in the AuroFlow AQ Supplemental Extraction Reagents (Catalog # FOOD-141301-200) kit:
 - a. 25 Large Extraction Bags
 - b. 9 Large AQ Powder Packs
 - c. 3 tubes of AQ Add-in Solution
3. Required materials, but not provided:
 - a. Strip Reader
 1. QuickStar Horizon Strip Reader (Catalog # FOOD-6006-01)
 - b. Recommended orbital shaker setup:
 1. Orbital shaker (VWR # 0027-142)
 2. Shaker platform (VWR # 97003-590)
 3. Stainless Steel Clamps (VWR # 14215-238)
 4. 1000 mL Plastic Beaker (VWR # 76266-414)
 - c. Microtube rack
 - d. Distilled or deionized water
 - e. Timer or watch
 - f. Single-channel pipettor(s)

1. 1000 μ L pipettor: used for 1000, 700, 600, and 300 μ L volume transfer (VWR # 89079-974)
2. 200 μ L pipettor: used for 100 and 150 μ L volume transfers (VWR # 89079-970)
3. 1000 μ L pipet tips (VWR # 89136-165)
4. 200 μ L pipet tips (VWR # 89136-161)
- g. Pipet controller
- h. 10 mL graduated serological pipets (VWR # 89130-898)
- i. 5 Transport Tube (VWR # 89005-596)
- j. Scale capable of measuring 50 grams – see Mycotoxin Handbook
- k. Grain mill / grinder – see Mycotoxin Handbook
- l. 250 mL graduated cylinder (VWR #65000-008)
- m. Storage bottle(s) for AQ Extraction Buffer (glass or plastic, VWR # 16157-282)
- n. Vortex mixer (VWR # 10153-838)
- o. Filter funnel (VWR # 414004-288)
- p. Conical tubes, 50 mL (VWR # 89174-474)
- q. Wide-mouth funnel (VWR # 414004-272)
- r. AuroFlow AQ Supplemental Extraction Reagents (Catalog # FOOD-141301-200)
4. Optional materials not provided:
 - a. QuickStar Horizon Printer (Catalog # FOOD-6007-01)

REVISION HISTORY

Effective: 09/29/2020