

Test Kit Instruction

December 16, 2020

NEOGEN **Reveal Q+ MAX for DON Using AccuScan Gold Reader**

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 the 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).

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1. GENERAL INFORMATION

The Reveal Q+ MAX for DON test method provided by the NEOGEN is a single-step lateral flow immunochromatographic assay based on a competitive immunoassay format. The test provides quantitative analysis for the presence of DON, using distilled or deionized water and a MAX 1-G50 extraction packet as an extraction solvent along with a DON-antibody particle complex coated test strip and the Neogen's AccuScan Gold Reader.

Approved Test Kit Information	
Test Kit Vendor:	NEOGEN 800/234-5333
Test Kit Name:	Reveal Q+ MAX for DON using AccuScan Gold Reader
Product Number:	8388
Effective Date of Instructions:	12/16/2020
Conformance Range:	0.50 – 30 ppm
Number of Analyses to Cover Conformance Range:	2
Type of Service:	Quantitative
Approved Commodities:	Wheat (including whole grain wheat flour, wheat middlings, wheat red dog, wheat flour 2 nd clear, and wheat screenings), corn (including dent or field corn, corn meal, corn flour, cracked corn, corn grits or polenta, and corn screenings), malted barley (including malted barley flour), corn/soy blend, sorghum, corn gluten meal, buckwheat, milled rice (including brewer's rice and glutinous rice), brown rice, oats (whole oats with hull), soybeans (including whole soybean and full-fat soy flour), soybean meal, pearled barley (including quick pearl barley)
Extraction method:	Blend 50 grams sample and contents of one MAX 1-G50 extraction additive packet in 250 mL of distilled or deionized water for 30 seconds.
Test Format:	Lateral Flow Strip
Detection Method:	NEOGEN AccuScan Gold Reader, Model #9595

2 PREPARATION OF TESTING MATERIALS

a. AccuScan Gold Reader Set-up.

- (1) Enter the lot-specific QR code by selecting Scan QR Code from the main screen.
- (2) Place the QR code into the white cartridge adapter labeled Cal/QR and insert the cartridge into the reader.
- (3) The valid code will be scanned by the reader and provide information on the lot number and expiry date. Verify this information is correct and then add the Lot ID to the reader by pressing Add Lot ID.
- (4) Return to the home screen and select the test strip icon.
- (5) Select Mycotoxin Q+ MAX for the Category.
- (6) Select the **Q+M DON ppm** test type.

Note: The AccuScan Gold reader needs to be calibrated every year or every 1,000 readings, whichever comes first. To do this, insert the QR Cartridge without a QR code into the reader to calibrate. Select Settings-> Run Diagnostics-> Calibrate Now-> Run Calibration.

3. SAMPLE PREPARATION AND EXTRACTION PROCEDURES

The sample to be tested should be collected and prepared according to accepted sampling techniques (see Mycotoxin Handbook).

Extraction Procedure:

- a. Weigh 50 ± 0.2 grams ground samples into a blender jar.
- b. Add the contents of one MAX 1-G50 extraction additive packet.
- c. Using a 250 mL graduated cylinder, add 250 mL of distilled or deionized water and close the jar securely to prevent spillage. Immediately shake the mixture by hand for few seconds to ensure all the sample is wet and there is no lump.
- d. Blend for 30 seconds. Do not allow the sample to settle.

- e. Using a disposable polyethylene transfer pipette, fill a 1.5 – 2 mL microcentrifuge tube with the foam from the blender jar.
- f. Centrifuge for 30 seconds at 6,000 RPM in a micro-centrifuge.
- g. The supernatant liquid is the **centrifuged extract** and is ready for analysis.
- h. Proceed to **Test Procedures** below.

4. TEST PROCEDURES

a. Analysis Procedure (0.50 – 5.0 ppm Quantitation Range)

- (1) Place the appropriate number of red sample dilution cups and clear sample cups for each test sample in the sample cup rack. Label cups if necessary.
- (2) Using a 1000 µL pipette, add 1000 µL of sample diluent to each red sample dilution cup.
- (3) Using a 100 µL pipette, add 100 µL of the **centrifuged extract** into each red dilution cup containing sample diluent. Mix by swirling with the pipette tip first and then by pipetting up and down at least 5 times.
- (4) Using a 100 µL pipette, transfer 100 µL of the diluted sample extract into a new clear sample cup.
- (5) Place a new Reveal Q+ MAX for DON test strip with the sample end down into the sample cup. Start timer and incubate for 3 minutes.
- (6) At the end of the 3 minutes incubation period, remove the test strip from the sample cup. Read the test strip within 30 seconds using Neogen's AccuScan Gold reader.

b. Diluted Sample Analysis Procedure (5.0 – 30 ppm Quantitation Range)

- (1) Using the **centrifuged extract** made in **Extraction Procedure** above, dilute the **centrifuged extract** six-fold with distilled or deionized water to prepare the **Diluted Extract**.

- (2) Using a 100 µL pipette, add 100 µL of **centrifuged extract** to 500 µL of distilled or deionized water (previously added using a 1000 µL adjustable pipette). This is **Diluted Extract**. Vortex for few seconds prior to the analysis.
- (3) Follow the same test procedure as described in “**a. Analysis Procedure (0.5 – 5.0 ppm Quantitation Range)**” except instead of 100 µL of the **centrifuged extract** use 100 µL of the **Diluted Extract**.
- (4) To obtain the final DON concentration in the original test sample must multiply the result of this Diluted Extract by 6. Results following this protocol are valid in the range of 5.0 – 30 ppm.

A final result (using 5.0 – 30 quantitation range) less than 3.5 ppm is indicative of a problem, and troubleshooting is needed. Verify the procedure is being followed properly. Perform the analysis using 0.5 – 5 ppm quantitation range (**Analysis Procedure**) above and only perform the analysis using 5.0 – 30 ppm quantitation range again if the value is greater than 5 ppm.

5. 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).

6. STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions.

Store test kit at room temperature (18-30°C, 64-86°F) when not in use, do not freeze. Test strips should remain capped in their original tubes until used to ensure optimal performance.

b. Precautions.

- (1) Do not use test kit components beyond the **expiration date**.
- (2) Ensure the device lot number and the curve details match the lot ID number selected on the reader. Failure to update the lot-specific QR code within the AccuScan Gold will cause inaccurate results.
- (3) The test strips must remain inside the stay-dry tube before use.
- (4) Store test kits at room temperature (18-30°C, 64-86°F) when not in use, do not freeze.

- (5) Treat all used liquids, including sample extract, and lab ware as if contaminated with DON. Gloves and other protective apparel should be worn at all times.
- (6) To avoid cross-contamination, use new pipette tip for each measurement.
- (7) Commodity extracts should have a pH of 7 – 8 before testing. Excessively acidic or alkaline samples should be adjusted. For instructions on adjusting pH contact a Neogen representative or Technical Services.

7. EQUIPMENT AND SUPPLIES

a. Materials provided in test kits.

- (8) 25 Reveal Q+ MAX for DON test strips; 25 red sample dilution cups
- (9) 25 clear sample cups; 2 bottles of sample diluent
- (10) Instructions for use

b. Materials required but not provided.

- (11) Timer (NEOGEN item #9426)
- (12) 100 µL pipette (NEOGEN item #9272- fixed, #9278-Basic fixed)
- (13) 1000 µL pipette (NEOGEN item #9337-fixed)
- (14) 100 –1000 µL adjustable pipette, (NEOGEN item #9290)
- (15) 100 µL pipette tips (NEOGEN item #9407-tip rack (96 tips), #9410-bag of 1000 tips, #9417-10 reload decks)
- (16) 1000 µL pipette tips (NEOGEN item #9464- bag of 1000 tips, #9487-tip rack (96 tips), #9292- tip rack (5 racks of 192 tips), #9293- bag of 1000 tips)
- (17) Microcentrifuge tubes (NEOGEN item #9372- 1.5 mL, #9172- 2 mL)
- (18) Microcentrifuge (NEOGEN item #9330)
- (19) Reveal sample rack. (NEOGEN item #9475)
- (20) AccuScan Gold Reader (NEOGEN item #9475)
- (21) Disposable polyethylene transfer pipettes, 1.5 mL (NEOGEN item #9328 or VWR item #470225-068-052).

- (22) Dispensing pump or graduated cylinder (NEOGEN item #9448, #9447)
- (23) Sample grinder (see FGIS Mycotoxin Handbook)
- (24) Agri-Grind grinder or equivalent (NEOGEN item #9401, see FGIS Mycotoxin Handbook)
- (25) Scale capable of weighing 50 grams (NEOGEN item #9427, see FGIS Mycotoxin Handbook)
- (26) Bottle, 1 Liter (NEOGEN item #9472)
- (27) Blender (NEOGEN item #9493)
- (28) MAX 1-G50 Aqueous Extraction packets (NEOGEN item #8089G)

8. REVISION HISTORY

Effective: 12/16/2020