

# Test Kit Instruction

March 2, 2018

## **NEOGEN CORPORATION** **REVEAL Q+ 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@ams.usda.gov](mailto:Ajit.K.Ghosh@ams.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 (816-891-8403 or [Patrick.J.McCluskey@ams.usda.gov](mailto:Patrick.J.McCluskey@ams.usda.gov)).

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## 1. GENERAL INFORMATION

The Reveal Q+ for DON test method provided by the Neogen Corporation 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 water as an extraction solvent along with a DON-antibody particle complex coated test strip and the Neogen AccuScan Gold Reader.

Approved Test Kit Information	
<b>Test Kit Vendor:</b>	<i>Neogen Corporation 800/234-5333</i>
<b>Test Kit Name:</b>	Reveal Q+ for DON using AS Gold
<b>Product Number:</b>	8385
<b>Effective Date of Instructions:</b>	3/02/2018
<b>Instructions Revision Number:</b>	1
<b>Conformance Range:</b>	0.50 – 30 ppm
<b>Number of Analyses to Cover Conformance Range:</b>	3
<b>Type of Service:</b>	Quantitative
<b>Supplemental Analysis:</b>	Yes
<b>Approved Commodities:</b>	Wheat (whole grain wheat flour, wheat middlings, wheat red dog, wheat flour 2nd clear, and wheat screenings), corn (dent or field corn, corn meal, corn flour, cracked corn, corn grits or polenta, and corn screenings), corn/soy blend, distillers dried grain with solubles (DDGS), soybeans (including whole soybean and full-fat soy flour), malted barley (including malted barley flour), buckwheat, brown rice, barley (with hull), and sorghum
<b>Extraction method:</b>	Shake 50 grams sample with 250 mL of deionized or distilled water by mechanical shaker (250 rpm) for 3 minutes
<b>Test Format:</b>	Lateral Flow Strip
<b>Detection Method:</b>	AccuScan Gold Reader, Model #9595

## 2. PREPARATION OF TESTING MATERIALS

### a. AccuScan Gold Reader Set-up.

The system provides an easy method to objectively read, store, and analyze results from Neogen's line of lateral flow strips.

**Note:** Please keep and store all packaging materials included in the kit for future storage.

- (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+ for the Category
- (6) Select the **Q+ DON** test type.

**Note:** The AccuScan Gold reader needs to be calibrated every year or every 1000 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.**

#### Reader Notes and Cautions:

- Ensure device is fully inserted into cartridge.
- Reading should be made between 3 and 4 minutes. Reading results after 4 minutes may be inaccurate due to over development of the device.

b. **Preparation of 1N Sodium Hydroxide (NaOH) Solution.**

**NOTE:** One can buy premade 1N NaOH from any commercial supplier (e.g. Sigma Aldrich catalog# 72082) or may prepare from solid sodium hydroxide pellets (Sigma Aldrich catalog# S8045) as described below:

- (1) Add slowly 4 grams of NaOH into 100 mL distilled or deionized water with stirring.
- (2) This solution should be used to adjust the pH of any sample extract that shows pH below 7.0
- (3) Label the container stating the name, date of preparation, and initials of technician that prepared the solution.
- (4) Store this solution at room temperature in a tightly closed container under fume hood.

**CAUTION! NaOH is corrosive. Addition of solid NaOH pellets into water is an exothermic reaction (produces heat). Stir constantly and add the NaOH slowly.**

c. **Preparation of 1N Hydrochloric (HCl) Acid Solution.**

**NOTE:** One can buy premade 1N HCl from any commercial supplier (e.g. Sigma Aldrich catalog# 38283) or may prepare from concentrated HCl (Sigma Aldrich catalog# 320331) as described below:

- (1) Add slowly 8.2 mL of 12.1N HCl (concentrated Hydrochloric Acid) into 91.8 mL distilled or deionized water with stirring.
- (2) This solution should be used to adjust pH of any sample extract that shows pH above 8.0
- (3) Label the container stating the name, date of preparation, and initials of technician that prepared the solution.
- (4) Store this solution at room temperature in a tightly closed container under a fume hood.

**CAUTION! HCl is corrosive. Addition of acid into water is an exothermic reaction (produces heat). Stir constantly and add the HCl slowly.**

### 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  $50 \pm 0.2$  grams ground samples into a Whirl-Pak bag.
- b. Using a 250 mL graduated cylinder, add 250 mL of distilled or deionized water and close the bag securely to prevent spillage.
- c. Shake vigorously by mechanical shaker (250 rpm) for 3 minutes. Allow the sample to settle for a minimum of 3 minutes.
- d. Filter about 2 to 3 mL of sample extract using a Neogen syringe filter. The filtrate should be a clean liquid free of any particulate. A second filtration should be done if filtrate is cloudy.
- e. For DDGS, check the pH of the filtered extract. For all other commodities proceed to step f.
  - (1) If the pH is not between 6.0 – 8.0 it needs to be adjusted.
  - (2) Using a disposable polyethylene transfer pipette, add one drop of 1N NaOH (sodium hydroxide) to the sample extract, vortex to mix, and check the pH.
  - (3) If the pH is still below 6.0, add another drop of 1N NaOH, mix, and check pH again. Continue this process until the pH falls between 6.0 and 8.0, then proceed to step f.
- f. Dilute the filtrate two-fold with distilled or deionized water. Using a 1000 microliter ( $\mu\text{L}$ ) pipette, add 1.0 mL of filtrate to 1.0 mL of distilled or deionized water. This is the **diluted filter extract**. Vortex for few seconds before the analysis.
- g. Proceed to **Test Procedures** below.

## 4. TEST PROCEDURES

### a. Analysis Procedure (0.5 – 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 **diluted filter extract** into each red dilution cup containing sample diluents. Mix by swirling with the pipette tip first and then by pipetting up and down at least 5 times, and transfer 100 µL into a new clear sample cup.
- (4) Place a new Reveal Q+ for DON test strip with the sample end down into the sample cup. Start timer and incubate for 3 minutes.
- (5) At the end of the 3 minutes incubation period, remove the test strip from the sample cup and read the test strip immediately (must be within one minute) using Neogen's AccuScan Gold reader.
- (6) Results following this protocol are valid in the range of 0.5 – 5 ppm. If the result is more than 5 ppm, run the test using extraction procedure for 5 –10 ppm quantitation range (Dilution A Protocol) below.

### b. Dilution A Protocol (5.0 – 10 ppm Quantitation Range)

- (1) Using the **diluted filtered extract** made in **Extraction Procedure** above, dilute the **diluted filtered extract** two-fold with distilled or deionized water to prepare the **Diluted Extract A**.
- (2) Using a 1000 µL pipette, add 1.0 mL of **diluted filtered extract** to 1.0 mL of distilled or deionized water. This is **Diluted Extract A**. 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 **diluted filter extract** use 100 µL of the **Diluted Extract A**.
- (4) To obtain the final DON concentration in the original test sample multiply the result of this Diluted Extract A with 2. Results following this protocol are valid in the range of 5.0 – 10 ppm.

A final result (using 5.0 – 10 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 (**Dilution A Protocol**) above and only perform the analysis using 5 – 10 ppm quantitation range again if the value is greater than 5 ppm. But if final result is more than 10 ppm, run the test using the extraction procedure for 10 – 30 ppm quantitation range (**Dilution B Protocol**) below.

### c. **Dilution B Protocol (10 – 30 ppm Quantitation Range)**

- (1) Using the **diluted filtered extract** made in **Extraction Procedure** above, dilute the **diluted filtered extract** 8-fold with distilled or deionized water to prepare the **Diluted Extract B**.
- (2) Using a 100 µL pipett, add 100 µL of **diluted filtered extract** to 700 µL (measured using 100–1000 µL variable volume pipet) of distilled or deionized water. This is **Diluted Extract B**. 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 diluted filter extract use 100 µL of the **Diluted Extract B**.
- (4) The result of this **Diluted Extract B** must be multiplied by 8 to obtain the actual DON concentration of the original test sample. Results following this protocol are valid in the range of 10 – 30 ppm

A final result (using 10 – 30 quantitation range) less than 7.0 ppm is indicative of a problem, and troubleshooting is needed. Verify the procedure is being followed properly. Perform the analysis using 5 – 10 ppm quantitation range and only perform the analysis using 10 – 30 ppm quantitation range again if the value is greater than 10 ppm.

### d. **Reading the Results**

- (1) The strips must be read immediately using Neogen’s AccuScan Gold reader to analyze test strip. Test results will be displayed and stored in the reader.
- (2) Fully insert the Reveal Q+ test strip into the cartridge adapter with the sample end first and results facing out.

## 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@udsa.gov](mailto:Patrick.J.McCluskey@udsa.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) 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.
- (4) To avoid cross-contamination, use new pipette tip for each measurement.

**7. EQUIPMENT AND SUPPLIES**

**a. Materials provided in test kits.**

- (1) 25 Reveal Q+ for DON test strips; 25 red sample dilution cups
- (2) 25 clear sample cups; 2 bottles of sample diluent
- (3) Instructions for use

**b. Materials required but not provided.**

- (1) Timer (Neogen item #9426)
- (2) 100 µL pipet (Neogen item #9272- fixed, #9278-Basic fixed)
- (3) 1000 µL pipet (Neogen item #9337-fixed)
- (4) 100–1000 µL Pipet, (Neogen item #9290)
- (5) 100 µL pipette tips (Neogen item #9407-tip rack (96 tips), #9410-bag of 1000 tips, #9417-10 reload decks)
- (6) 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)
- (7) Stomacher type sampling bag (Neogen item #9736)
- (8) Filter syringe (Neogen item #9420)
- (9) Reveal sample rack. (Neogen item #9475)
- (10) Reveal AccuScan Gold Reader (Neogen item #9595)

- (11) Disposable polyethylene transfer pipettes; Dispensing pump or graduated cylinder (Neogen item #9448, #9447)
- (12) Agri-Grind grinder or equivalent (Neogen item #9427)
- (13) Scale capable of weighing 50 grams (Neogen item #9427)
- (14) Bottle, 1 Liter (Neogen item #9472)
- (15) 1N NaOH (Sigma Aldrich #72082), or NaOH pellets (Sigma Aldrich #S8045)
- (16) 1N HCl (Sigma Aldrich #38283), or concentrated HCl (Sigma Aldrich #320331)

## **8. REVISION HISTORY**

### **Revision 0 (12/05/2017)**

### **Revision 1 (3/02/2018)**

In this revision, corn/soy blend, distillers dried grain with solubles (DDGS), soybeans (including whole soybean and full-fat soy flour), malted barley (including malted barley flour), buckwheat, brown rice, barley (with hull), and sorghum were added as additional commodities, and the test procedures of these additional commodities were also incorporated.