

Test Kit Instruction

March 2, 2018

NEOGEN REVEAL Q+ FOR DON USING ACCUSCAN PRO READERS

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 (816-891-8403 or Patrick.J.McCluskey@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 Pro Reader.

Approved Test Kit Information	
Test Kit Vendor:	<i>Neogen Corporation 800/234-5333</i>
Test Kit Name:	Reveal Q+ for DON using AS Pro
Product Number:	8385
Effective Date of Instructions:	03/02/2018
Instructions Revision Number:	1
Conformance Range:	0.50 – 30 ppm
Number of Analyses to Cover Conformance Range:	3
Type of Service:	Quantitative
Supplemental Analysis:	Yes
Approved Commodities:	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
Extraction method:	Shake 50 grams sample with 250 mL of deionized or distilled water by mechanical shaker for 3 minutes.
Test Format:	Lateral Flow Strip
Detection Method:	AccuScan Pro Reader, Model #9565

2. PREPARATION OF TESTING MATERIALS

a. AccuScan Pro 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) Set-up: Press the Home icon at any time to return to the main screen.



(a) Plug the outlet of the cord into the power source. Plug the small end of the power cord into the reader.

(b) This will turn the reader on. To turn the reader off, unplug the reader.

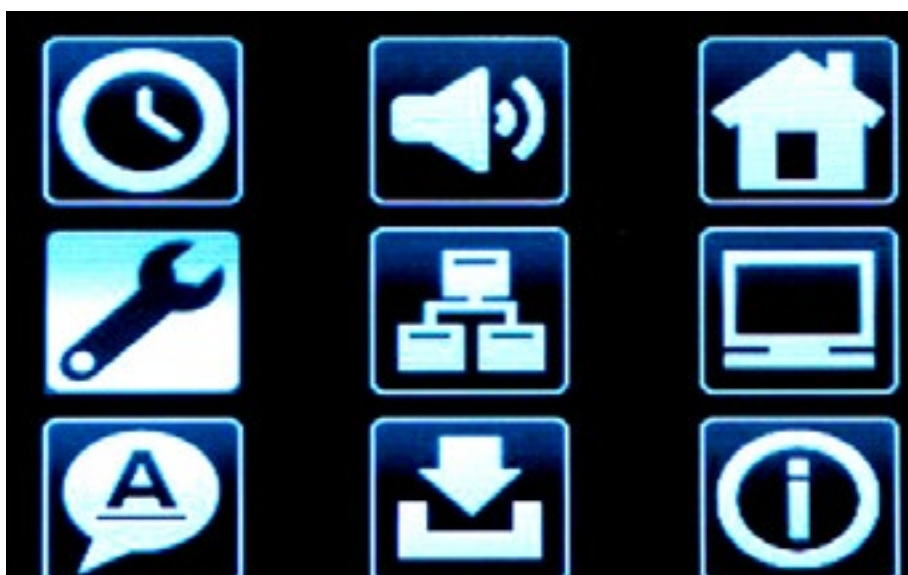
Note: To place in the power down mode, press the small power icon in the lower right corner of the Home screen. The screen will turn white and the reader may then be unplugged.

(c) Plug the USB key into the USB port on the side of the reader.

(2) Settings.



Press the designated icon to set time, date, volume, remote connection, language setting, and allows the user to review current information.



(3) QR Codes.



The reader is capable of reading information from predefined QR codes. Disposable QR codes containing lot specific information are included in each Reveal Q+ kit.



- (a) Whenever a new lot is obtained, and before you run a test, you must verify the QR codes. Simply place the QR code card into the QR code cartridge. From the Home screen, select the QR code icon and place the QR code cartridge containing the card in the reader. The specific information automatically will be downloaded into the reader.
- (b) The specific QR code cartridges are entered into the reader to identify unique users with a five digit user ID code.

Note: Technicians must update or verify reader information (lot number of test strips in use) before official testing. If needed refer to the AccuScan Pro manual for more detailed instructions.

(4) Run test protocol.



- (a) Touch the **Run test** icon, which looks like a test strip, on the Home screen.
- (b) The test category will display in the first box and the test type will display in the second box. The reader will default to the last test analyzed.
- (c) Select the appropriate test category by touching the desired option.
- (d) Touch the green check mark to confirm the selection.

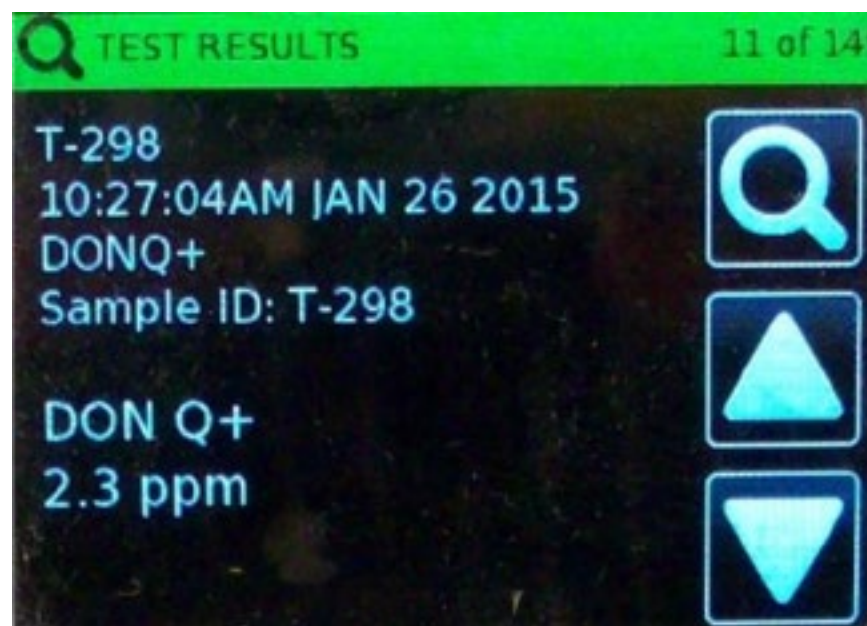
- (e) Touch the **Test type** icon. Select the desired test by touching the name of the test (e.g., Q+ DON). To scroll through the test options, use the white up and down arrows. Touch the green check mark to confirm the selection.
- (f) Insert the test strip to begin interpreting the test or enter the sample ID by touching the **Sample ID** button.

NOTE: Strips may be tested without a sample ID. To do this, insert the test strip cartridge containing the test strip into the reader square end first. The reader automatically will begin analyzing the strip.

- (g) If entering a sample ID, type the ID in and press the green check mark to continue.
- (h) Insert the test strip into the appropriate strip cartridge. Insert the cartridge containing the strip into the slot on the lower left portion of the reader. The reader automatically will begin analyzing the strip.

(5) Test Results.

- (a) For a quantitative test, a numerical value will be displayed.
- (b) Touch the screen to view additional test information such as test line and control line ratios.



- (c) To print, select the **Print** icon. A report will automatically be sent to the attached printer.
- (d) To begin/run a new test, select the **"New test"** icon. This will return the user to the test setup screen.

(6) Reader Notes and Cautions.

- Ensure device is fully inserted into cartridge
- Removing the cartridge prior to completion can result in invalid readings
- Reading should be made between 3 and 4 minutes. Reading results after 4 min 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 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 concentrated acid into water is an exothermic reaction (produces heat). Stir constantly and add 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 ± 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 of the filtered extract is not in 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 (μL) pipet, 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**.

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 pipet, add 1000 µL of sample diluent to each red sample dilution cup.
- (3) Using a 100 µL pipet, 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 with the 100 µL pipet 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 minute 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 Pro 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 – 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 pipet, 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) The result of this **Diluted Extract A** must be multiplied by 2 to obtain the final DON concentration of the original test sample. Results following this protocol are valid in the range of 5 – 10 ppm.

A final result (using 5 – 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. However, if 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 pipet, 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 Pro 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.
- (3) Insert the cartridge with test strip side up into the AccuScan Pro.
- (4) The reader will automatically begin analyzing the cartridge.

5. **REPORTING AND CERTIFYING TEST RESULTS**

Refer to the current 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).

6. STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions.

Store kit components at room temperature (18-30°C, 64-86°F) to ensure full shelf life. 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 Pro will cause inaccurate results.
- (3) The test strips must remain inside the stay-dry tube before use.
- (4) Store test kit 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 clean glassware for each sample and thoroughly wash all glassware between samples.

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 variable 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) Reveal sample rack. (Neogen item #9475)
- (9) Reveal AccuScan Pro Reader (Neogen item #9565)
- (10) Disposable polyethylene transfer pipettes; Dispensing pump or graduated cylinder (Neogen item #9448, #9447)
- (11) Agri-Grind grinder or equivalent (Neogen item #9427)
- (12) Scale capable of weighing 50 grams (Neogen item #9427)
- (13) Bottle, 1 Liter (Neogen item #9472)
- (14) 1N NaOH (Sigma Aldrich #72082) or NaOH pellets (Sigma Aldrich #S8045)
- (15) 1N HCl (Sigma Aldrich #38283) or concentrated HCl (Sigma Aldrich #320331)

8. REVISION HISTORY

Revision 0 (7/07/2017)

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.