

NEOGEN
REVEAL Q+ FOR DON
USING ACCUSCAN PRO READER

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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.

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 current Mycotoxin Handbook for information on use of this test kit in official inspections including sampling, general sample preparation (e.g., grinding and dividing), reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey of Policies, Procedures, and Market Analysis Branch (PPMAB) of the Field Management Division by phone at 816-659-8403 or email at Patrick.J.McCluskey@usda.gov.

Approved Test Kit Information

Test Kit Vendor:	<i>Neogen Corporation 800/234-5333</i>
Test Kit Name:	Reveal Q+ for DON
Product Number:	8385
Effective Date of Instructions:	03/06/2016
Instructions Revision Number	0
Conformance Range:	0.5 – 5 ppm
Number of Analyses to Cover Conformance Range:	1
Type of Service:	Quantitative
Supplemental Analysis:	Yes
Approved Commodities:	Wheat, corn, barley, malted barley, dried distillers grains with solubles (DDGS), oats, rice, corn gluten meal and wheat middlings
Extraction method:	Shake 50 grams sample in 250 mL of deionized water for 3 minutes.
Test Format:	Lateral Flow Strip
Detection Method:	AccuScan Pro Reader only

PREPARATION OF TESTING MATERIALS

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.



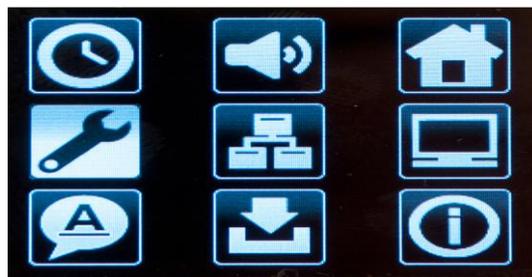
USB, Ethernet, and power cord ports



Start up screen

(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: 

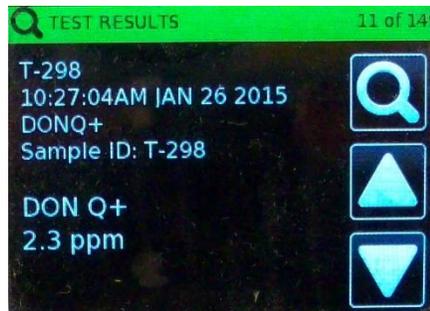
- a. Touch the **Run test** icon, which looks like a test strip, on the **Home screen**.
- b. The test category will display in the first box and the test type will display in the second box. The reader will default to the last test analyzed.
- c. Select the appropriate test category by touching the 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.

Reader Notes and Cautions:

- a. Ensure device is fully inserted into cartridge
- b. Removing the cartridge prior to completion can result in invalid readings
- c. Reading should be made between 3 and 4 minutes. Reading results after 4 min may be inaccurate due to over development of the device.

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.

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.

SAMPLE PREPERATION AND EXTRACTION PROCEDURES

a. Sample Preparation for wheat, corn, barley, corn gluten meal (CGM), distillers dried grains with solubles (DDGs), malted barley, oats, rice, and wheat middlings

The sample to be tested should be collected according to accepted sampling technique.

- (1) Obtain a representative sample

- (2) Grind the sample so that at least 95% of the ground material passes through a 20 mesh sieve, about the particle size of fine instant coffee.

b. Extraction Procedure for wheat, corn, barley, oats, rice, and wheat middlings

- (1) Weigh 50 ± 0.2 grams ground sample into a whirl pak bag.
- (2) Add 250 mL of distilled or deionized water and close the bag securely to prevent spillage.
- (3) Shake vigorously by mechanical shaker (250 rpm) or by hand with similar shaking action for 3 minutes. Allow the sample to settle for 3 minutes.
- (4) Filter about 3 mL of sample extract using a Neogen syringe filter and collect the filtrate.
- (5) Dilute the filtrate by two fold with distilled or deionized water. For example, add 1 mL of filtrate to 1 mL of distilled or deionized water. This is the diluted filter extract and ready for the analysis.

c. Extraction Procedure for Corn gluten meal (CGM), distillers dried grains with solubles (DDGs), and malted barley

- (1) Weigh 50 ± 0.2 grams ground samples into a blender cup.
- (2) Add 250 mL of distilled or deionized water and close the bag securely to prevent spillage.
- (3) Shake vigorously by mechanical shaker (250 rpm) or by hand with similar shaking action for 3 minutes. Allow the sample to settle for 3 minutes.
- (4) Filter about 3 mL of sample extract using a Neogen syringe filter and collect the filtrate.
- (5) Check the pH of the filtered extract using pH paper (Neogen item #9478) or equivalent. A pH meter may also be used in place of pH paper if available.

If the pH is not between 7.0 and 8.0, and if it is below 7.0, it needs to be adjusted.

- a. 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.
- b. If pH is still below 7.0, add another drop of 1N NaOH, mix, and check pH again. Continue this process until pH falls between 7.0 and 8.0, and then proceed step 6.
- (6) Dilute the filtrate by two fold with distilled or deionized water. For example, add 1 mL of filtrate to 1 mL of distilled or deionized water. This is the diluted filter extract and ready for the analysis.

TEST PROCEDURES

a. Analysis Procedure

- (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 single-channel pipettor with a new pipette tip, add 1000 microliters (μL) of sample diluent to each red sample dilution cup.
- (3) Using a new pipette tip, add 100 μL of the diluted filter extract into each red dilution cup with sample diluents. Mix by swirling with the pipette tip and then by pipetting up and down 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 minute incubation/development period, remove the test strip from the sample cup. Read the test strip within one minute using only Neogen's AccuScan Pro Reader.

b. Reading the Results

- (1) The strips must be immediately read using Neogen's AccuScan Pro Reader to analyze test strip. Test results will be displayed and stored in the reader.
- (2) Fully inserted 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) The AccuScan Pro reader will analyze the test strip and test results will be displayed and stored in the reader.

SUPPLEMENTAL ANALYSIS

Supplemental analysis (wheat only) is a procedure followed when a result is observed above the upper limit of the concentration range used in GIPSA's test kit performance evaluation. The range for performance evaluation of quantitative DON test kits is 0.5 – 5.0 ppm. Therefore, supplemental analysis would be performed for a result above 5.0 ppm. In supplemental analysis, the extract is diluted so the resulting concentration is between the lower and upper limits of the test kit evaluation range (i.e., 0.5 – 5.0 ppm for DON), and a correction for dilution is applied to derive at the final result. Supplemental analysis is performed only at the request of the applicant.

Supplemental Dilution Procedure

- (a) Combine 100 µL diluted filtered extract with 100 µL distilled or deionized water.
- (b) Mix by swirling or pipetting up and down 5 times. This is the diluted filtered extract used for supplemental analysis.
- (c) Refer to “**Test Procedures**” section for analysis.
- (d) Read and record results on the work record, then multiply the analytical results obtained from the AccuScan reader by 2 to obtain the actual DON concentration of the original test sample (show all results on the work record).

Example: If the diluted filtered extract is diluted 1:1 (v/v) using extractions solvent, the dilution factor is two (2).

AccuScan Pro reader supplemental analysis results:	4.0 ppm
Multiplied by the dilution factor	<u>x 2</u>
Sample results - TOTAL:	8.0 ppm

A final result less than 3.5 ppm is indicative of a problem, and troubleshooting is needed. Verify the procedure is being followed properly. Perform the procedure for the Diluted Extract (non-supplemental analysis) and only perform the supplemental analysis again if the value is greater than 5.0 ppm.

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@usda.gov).

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 QC code within the AccuScan Pro reader 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.

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); 100 µL pipettor (Neogen item #9272, #9278)
- (2) 100 µL pipette tips (Neogen item #9407, #9410, #9417); 500 µL pipettor (Neogen item #9291, #9336)
- (3) 200-1000 µL pipette tips (Neogen item #9464, #9487, #9292, #9293); Sample collection cups with lids. (Neogen item #9428); Reveal sample rack. (Neogen item #9475)
- (4) Reveal AccuScan Pro Reader (Neogen item #9565)
- (5) Disposable polyethylene transfer pipettes; Dispensing pump or graduated cylinder (Neogen item #9448, #9447)
- (6) Agri-Grind grinder or equivalent (Neogen item #9427); Scale capable of weighing 5 – 50 grams (Neogen item #9427)
- (7) Bottle, 1 Liter (Neogen item #9472)

REVISION HISTORY

Revision 0 (03/06/2016)