

NEOGEN
REVEAL Q+ FOR DON

Table of Contents	Page
GENERAL INFORMATION:	1
PREPARATION OF TESTING MATERIALS:.....	2
EXTRACTION PROCEDURES:.....	2
TEST PROCEDURES:.....	3
SUPPLEMENTAL ANALYSIS:.....	3
REPORTING AND CERTIFYING TEST RESULTS:.....	4
STORAGE CONDITIONS AND PRECAUTIONS:.....	5
EQUIPMENT AND SUPPLIES:.....	5
REVISION HISTORY:.....	6

GENERAL INFORMATION

Reveal Q+ for DON is a direct competitive quantitative lateral flow assay which allows the user to determine concentrations of deoxynivalenol (DON) in parts per million (ppm). DON is extracted from ground samples with water by shaking followed by filtration. The extracted toxin in the filtrate is then sampled and mixed with a diluent buffer. The mixed solution is transferred to a reaction cup. Once the lateral flow device is inserted, free toxin and toxin-gold complex compete for antibody binding sites on the lateral flow device. A line develops as a result of the presence of toxin-gold complex in inverse proportion to the toxin concentration. The intensity of the line in comparison to a control line is calculated using the AccuScan Gold reader and concentration of DON in the sample is then calculated from this data.

The instructions presented in this document cover only the procedure for performing the analytical test. 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 policies and/or instructions issued by the Policies, Procedures, and Market Analysis Branch (PPMAB) of the Field Management Division for information on use of this test kit in official FGIS 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 PPMAB by phone at 816-659-8403 or email at Patrick.J.McCluskey@usda.gov.

Approved Test Kit Information

Test Kit Vendor:	Neogen Corporation 800-234-5333
Test Kit Name:	Reveal Q+ for DON
Product Number:	8385
Effective Date of Instructions:	01/07/2015
Instructions Revision Number:	2
Conformance Range:	0.5 – 5.0 ppm
Number of Analyses to Cover Conformance Range:	1
Type of Service:	Quantitative
Supplemental Analysis:	Yes, for corn and wheat only
Approved Commodities:	Wheat, corn, barley, corn gluten meal (CGM), dried distillers grains with solubles (DDGs), malted barley, oats, rice, wheat bran, wheat flour and wheat middlings
Extraction method:	Shake 50 gram sample with 250 mL of deionized or distilled water for 3 minutes using mechanical shaker at 250 rpm (or shake vigorously by hand with similar shaking motion).
Test Format:	Lateral Flow Strip

Detection Method:

AccuScan Gold, Model #9595

PREPARATION OF TESTING MATERIALS

AccuScan Gold Reader Setup

- (1) Enter the lot-specific QR code by selecting the Scan QR code icon on the reader. Place the QR code into the cartridge and insert the cartridge into the reader.
- (2) Click Add Lot ID to store the QR Code. Confirm that curve set on the screen matches what is on the inside of the DON Q+ box or on the certificate of analysis (CoA).
- (3) Return to the main screen and select the test strip icon. Select Mycotoxin Q+ in category, and then select DON Q+ in test type.
- (4) Click Run Test and verify the correct Lot ID is selected, and then select OK.



EXTRACTION PROCEDURES

- (1) Combine 50 ± 0.2 grams of ground sample with 250 mL of distilled or deionized water in a whirl pack bag.
- (2) Shake vigorously for 3 minutes by mechanical shaker (250 rpm) or by hand with similar shaking action.
- (3) Filter about 5 mL of the extract through a Neogen syringe filter. Dilute the filtered extract by 1:1 with distilled or deionized water. For example, add 1 mL of filtered extract to 1

mL of distilled or deionized water, and vortex (10 seconds). This is the **diluted filtered extract** and ready for test.

TEST PROCEDURES

- (1) Place the appropriate number of red sample dilution cups and clear sample cups into a sample cup rack. Label cups if necessary.
- (2) Add 1000 μ L of sample diluent to each red sample dilution cup.
- (3) Add 100 μ L of diluted filtered extract to a red dilution cup with sample diluent. Mix first by swirling with the pipette tip and then pipetting up and down 5 times.
- (4) Transfer 100 μ L of diluted sample extract into a new clear sample cup.
- (5) Place a new Reveal Q+ for DON test strip with the sample end down into the sample cup and develop for 3 minutes.
- (6) Remove the strip from the sample cup after it has developed for 3 minutes, and read the strip immediately in AccuScan Gold reader.

NOTES:

- Fully insert the Reveal Q+ test strip into the black cartridge adapter with the sample end first and the results facing out.
- Insert the cartridge with test strip side up. Ensure device is fully inserted into cartridge.
- Strip must be read within 1 minute of completion of the 3 minute incubation. Readings after 4 minutes may be inaccurate due to over-development of the device.



SUPPLEMENTAL ANALYSIS

Supplemental analysis (for wheat and corn 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 deoxynivalenol test kits is 0.5 – 5.0ppm. 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), and a correction for dilution is applied to derive at the final result. Supplemental analysis is performed only at the request of the applicant.

a. Supplemental Analysis Procedure

- (1) Dilute the diluted filtered extract that tested above 5 ppm in distilled or deionized water. In a separate tube combine 1 mL of diluted filtered extract with 1 mL of distilled or deionized water for a dilution factor of 2.
- (2) Vortex and proceed to the test procedure

b. Test Procedure

- (1) Place the appropriate number of red sample dilution cups and clear sample cups into a sample cup rack.
- (2) Using a pipettor and new tip add 1000 µL of sample diluent to each red dilution cup.
- (3) Using a new tip add 100 µL of the diluted filtered extract from above to the red dilution cup. Mix first by swirling with the pipette tip and then pipetting up and down 5 times.
- (4) Transfer 100 µL into a new clear sample cup. Repeat steps 3 & 4 for each separate sample.
- (5) Place a new Q+ DON test strip with the sample end down into the sample cup.
- (6) Allow the strip to develop for 3 minutes.
- (7) Remove strip at 3 minutes and read the results immediately with Neogen's AccuScan Gold reader.

c. Interpreting Results

Read test results and multiply all results by 2 (two) to calculate the actual value for a 1 to 1 dilution.

Example: AccuScan Gold reader results:	4.0 ppm
Times dilution factor:	$\times 2$
TOTAL:	8.0 ppm

A final result less than 3.5 ppm using the supplemental analysis is indicative of a problem, and troubleshooting is needed. Verify the procedure is being followed properly. Perform the procedure for the sample 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 instructions issued by the Policies, Procedures, and Market Analysis Branch of the

Field Management Division for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-659-8403 or Patrick.J.McCluskey@udsa.gov).

STORAGE CONDITIONS AND PRECAUTIONS:

a. Storage Conditions

Store test 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) The test strips must remain inside the stay-dry tube before use.
- (2) Store test kit at room temperature (18-30°C, 64-86°F) when not in use, do not freeze.
- (3) Do not use kit components beyond expiration date.
- (4) Treat all used liquids, including sample extract, and labware as if contaminated with DON. Gloves and other protective apparel should be worn at all times.
- (5) To avoid cross-contamination, use clean glassware for each sample, and thoroughly wash all glassware between samples.

EQUIPMENT AND SUPPLIES

a. Materials Provided

- (1) 25 Reveal Q+ for DON test strips
- (2) 25 red sample dilution cups
- (3) 25 sample cups
- (4) 2 bottles of sample diluent
- (5) Instructions for use

b. Materials Recommended But Not Provided

- (1) Distilled or deionized water
- (2) Sample collection cups with lids (Neogen item #9428)

- (3) Agri-Grind grinder or equivalent (Neogen item #9401, #9453)
- (4) Scale capable of weighing 5-50g \pm 0.1g (Neogen item #9427)
- (5) Timer (Neogen item #9426)

- (6) Reveal sample cup rack (Neogen item #9475)
- (7) Reveal AccuScan Pro Reader (Neogen item #9565)
- (8) Reveal AccuScan Gold Reader (Neogen item #9595)
- (9) Dispensing pump or graduated cylinder (Neogen item #9448, #9447)
- (10) Neogen syringe filter (Neogen item #9420)
- (11) Sample collection tubes with caps (Neogen item #9421, #9421B)
- (12) Pipettor, 100 μ L (Neogen item #9272, #9278)
- (13) Pipette tips, 100 μ L (Neogen item #9407, #9410, #9417)
- (14) Pipettor, 500 μ L or 1000 μ L (Neogen item #9335)
- (15) Pipette tips, 200-1000 μ L (Neogen item #9464, #9487)

REVISION HISTORY

Revision 2 (01/07/2015)

- Correct Acronym of Policies, Procedures, and Market Analysis Branch (PPMAB) has been used.
- Phone number of Patrick McCluskey (816-659-8403) has been corrected.

Revision 1 (11/07/2014)

- Two additional commodities were approved (wheat bran and wheat flour) for REVEAL Q+ FOR DON test. Test procedure for these additional commodities has been incorporated in this revision.
- Removed Carl Jackson as a contact.
- Added Dr. Ajit Ghosh as contact for the Technology and Science Division.

Revision 0 (07/31/2014)

- Instruction was available but not posted on FGIS website.