

# Test Kit Instruction

June 20, 2018

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## **NEOGEN REVEAL Q+ FOR FUMONISIN 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 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 of PPMB by phone at 816-659-8403 or email at [Patrick.J.McCluskey@ams.usda.gov](mailto:Patrick.J.McCluskey@ams.usda.gov).

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## 1. GENERAL INFORMATION

The Reveal Q+ for Fumonisin 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 fumonisins in parts per million (ppm) using a fumonisin-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 Fumonisin
<b>Product Number:</b>	8885
<b>Effective Date of Instructions:</b>	06/20/2018
<b>Conformance Range:</b>	0.50 – 30 ppm
<b>Number of Analyses to Cover Conformance Range:</b>	2
<b>Type of Service:</b>	Quantitative
<b>Approved Commodities:</b>	Corn (including dent or field corn, corn meal, corn flour, cracked corn, corn grits or polenta, and corn screenings), dried distillers grains with solubles (DDGS), corn gluten meal, flaking corn grits, milled rice (including brewer's rice and glutinous rice), rough rice, and wheat (including whole grain wheat flour, wheat middlings, wheat red dog, wheat flour 2nd clear, and wheat screenings)
<b>Extraction method:</b>	Shake vigorously 50 gram sample with 250 mL of 65% ethanol / 35% distilled or deionized water (v/v) for 3 minutes
<b>Test Format:</b>	Lateral flow strip
<b>Detection Method:</b>	AccuScan Gold reader, Mode /#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.

- (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 expiration date. Verify if 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) Touch the mycotoxin category.
- (6) Select the Q+ for Fumonisin test type.
- (7) Ensure that the correct lot number appears on the screen for the lot that is being used.

### 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 (measured using a 250 mL graduated cylinder) 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 prepared from concentrated HCl (Sigma Aldrich catalog #320331) as described below.

- (1) Using a 10 mL graduated cylinder measure 8.2 mL of 12.1N HCl (concentrated Hydrochloric acid) and add slowly into 91.8 mL (measured with a 250 mL graduated cylinder) distilled or deionized water with stirring.
- (2) This solution should be used to adjust the 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 fume hood.

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

3. **SAMPLE PREPARATION AND EXTRACTION PROCEDURES**

a. **Sample Preparation.**

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

b. **Preparation of Extraction Solvent: 65%ethanol/35%water (v/v)**

- (1) Using a 1000 mL graduated cylinder, measure 650 mL of ethanol and carefully transfer into a clean 1000 mL bottle.
- (2) Using a 500 mL graduated cylinder, measure 350 mL of distilled or deionized water and add into the bottle containing ethanol. Shake until completely mixed.
- (3) Label the container stating the mixture contained, date of preparation, and initial of the analyst who prepared the solvent.
- (4) Store the solvent in a tightly closed container at room temperature until needed.

c. **Extraction Procedures**

- (1) Transfer  $50 \pm 0.2$  g of ground sample into a Whirl Pak bag.
- (2) Measure 250 mL of extraction solvent using a 250 mL graduated cylinder and

add to the Whirl-Pak bag. Securely close the bag.

- (3) Shake vigorously by mechanical shaker (250 rpm) or by hand with similar shaking action for 3 minutes.
- (4) Allow the sample to settle for 1-2 minutes. Then filter 3 – 5 mL of the extract using a filter syringe (Neogen item #9420) into a clean sample collection tube and labeled with the sample identification.
- (5) After collecting the filtrate (filtered extract), dispose of the filter syringe and ground material according to waste disposal guidelines. Set the filtrate aside for sample analysis.
- (6) This is the **filtered sample extract** and is ready for analysis.
- (7) For DDGS and corn gluten meal, check the pH of the **filtered sample extract**.
  - i. If the pH is not between 7.0 – 8.0 it needs to be adjusted. pH is typically low for these commodities.
  - ii. 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.
  - iii. If pH is still below 7.0, add another drop of 1N NaOH, mix, and check pH again. Continue this process until the pH falls between 7.0 and 8.0.

#### 4. TEST PROCEDURES

##### 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 single-channel 100 µL pipettor with a new pipette tip, add 200 microliters (µL) of sample diluent to each red sample dilution cup. This will require pipetting two times.
- (3) Using a new pipette tip and a 100 µL pipettor, add 100 µL of **filtered sample extract** into each red dilution cup containing 200 µL sample diluent. Mix by swirling with the pipette tip and then by pipetting up and down 5 times.

Note: Prepare the above red cup dilutions immediately before testing.

- (4) Using a 100 µL pipettor, transfer 100 µL of diluted sample extract into a new clear sample cup.

- (5) Place a new Reveal Q+ for Fumonisin test strip with the sample end down into the sample cup. Start timer and incubate for 6 minutes.
- (6) At the end of the 6 minutes incubation/development period, remove the test strip from the sample cup. Read the test strip within one minute using only Neogen's AccuScan Gold Reader.

#### **Analysis Procedure (5.0 – 30 ppm Quantitation Range)**

- (1) Using the filtered sample extract made in Extraction Procedure above, dilute the filtered sample extract 7-fold with 65% ethanol to prepare Diluted Extract A.
- (2) Using a 100 µL pipette, add 100 µL of **filtered sample extract** to 600 µL (measured using 100 – 1000 µL variable volume pipette) of 65% ethanol. This is **Diluted Extract A**.
- (3) Following the same test procedure as described in “a. Analysis Procedure (0.50 – 5.0 ppm Quantitation Range)” except instead of 100 µL of the filtered sample extract use 100 µL of the **Diluted Extract A**.
- (4) The result of this Diluted Extract A must be multiplied by 7 to obtain the actual DON concentration of the original test sample. Results following this protocol are valid in the range of 5 – 30 ppm.

A final result (using 5 – 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 0.50 – 5 ppm quantitation range again if the value is greater than 5 ppm.

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

- (1) The strips must be read using Neogen's AccuScan Gold Reader to analyze test strip. Test results will be displayed and stored in the reader.
- (2) Reading should be made between 6 and 7 minutes. Reading results after 7 minutes may be inaccurate due to over development of the device and should not be reported.
- (3) Fully insert the Reveal Q+ test strip into the reader specific black cartridge adapter with the sample end first and results facing out.
- (4) Insert the cartridge with test strip side up into the AccuScan Gold Reader.
- (5) The reader will automatically begin analyzing the cartridge and the test result will be displayed and stored in the reader.

## 5. 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@ams.udsa.gov](mailto:Patrick.J.McCluskey@ams.udsa.gov)).

## 6. STORAGE CONDITIONS AND PRECAUTIONS

### a. Storage Conditions.

Store the 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) Test strip development times, other than those specified in Test Procedures section, may give inaccurate results.
- (3) Treat all used liquids, including sample extract, and labware as if contaminated with Fumonisin, gloves and other protective apparel should be worn at all times.
- (4) To avoid cross-contamination, use new tip for each measurement.
- (5) Ensure the device, lot number and curve details match the lot ID number selected on the reader. Failure to update the lot-specific QR code within the AccuScan Gold Reader will cause inaccurate results.

## 7. EQUIPMENT AND SUPPLIES

### a. Materials provided in test kits.

- (1) 25 Reveal Q+ for Fumonisin test strips.
- (2) 25 red sample dilution cups.
- (3) 25 clear sample cups.
- (4) 1 bottle of sample diluent.

### b. Materials required but not provided.

- (1) Timer (Neogen item #9426)



- (2) Reveal Sample rack (Neogen item #9475)
- (3) 100 µL pipettor (or equivalent) with pipette tips.
- (4) Sample collection cups with lids. (Neogen item #9428),
- (5) Reveal AccuScan Gold Reader (Neogen item #9595)
- (6) Disposable polyethylene transfer pipettes.
- (7) 65% Ethanol, reagent grade or better (Neogen item #8073, #8074)
- (8) Dispensing pump or graduated cylinder. (Neogen item #9448, #9447)
- (9) Filter syringe (Neogen item #9420)
- (10) Sample grinder
- (11) Scale capable of weighing 5 – 50 grams.
- (12) Bottle, 1 Liter. (Neogen item #9472)
- (13) pH paper (Neogen item #9478)
- (14) Sodium Hydroxide pellets (NaOH) Sigma Aldrich Catalog #S8045

## **8. REVISION HISTORY**

### **8885 Effective 6.20.2018**

The test kit was recertified and valid for another three years until 6/28/2021.

### **8885 Effective 06/27/2017**

The requirement for grinding samples so that at least 95% passes through a #20 sieve was removed from section 3, Sample Preparation and Extraction Procedures. The sample to be tested should be collected and prepared according to the Mycotoxin Handbook.

### **8885 Effective 09/24/2015**

Corn gluten meal, corn grits, dried distillers grains with solubles (DDGS), brewer's rice, rough rice, and wheat were approved as additional commodities. Test procedure for these additional commodities are incorporated in this revision.