

# Test Kit Instruction

May 29, 2018

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## **NEOGEN REVEAL Q+ FOR AFLATOXIN 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 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 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@ams.usda.gov](mailto:Patrick.J.McCluskey@ams.usda.gov).

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## 1. GENERAL INFORMATION

The REVEAL Q+ FOR AFLATOXIN 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 aflatoxins, using a 65% ethanol/35% water (v/v) extraction solvent along with an aflatoxin-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 Aflatoxin
<b>Product Number:</b>	8085
<b>Effective Date of Instructions:</b>	05/29/2018
<b>Conformance Range:</b>	5.0 – 300 ppb
<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), flaking corn grits, corn germ meal, corn gluten meal, corn/soy blend, corn starch, distillers dried grains with solubles (DDGS), milled rice (including brewer's rice and glutinous rice), popcorn, rough rice, sorghum, and wheat (including whole grain wheat flour, wheat middlings, wheat red dog, wheat flour 2nd clear, and wheat screenings).
<b>Extraction method:</b>	Vigorously shake 50 gram sample with 125 mL of 65% ethanol/35% distilled or deionized water 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.

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

### 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 6 and 7 minutes. Reading results after 7 minutes may be inaccurate due to over development of the device.
- The strips must be read using Neogen's Reveal AccuScan Gold Reader.

### 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 may prepare 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 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.**

**d. Preparation of Extraction Solvent: Ethanol/Water (65/35, 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.

### 3. SAMPLE PREPARATION AND EXTRACTION PROCEDURES

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

#### a Standard Extraction Procedure

- (1) Transfer 50 g ( $\pm$  0.2) of ground sample into the whirl-pak bag.
- (2) Measure 125 mL of extraction solvent using a 250 mL graduated cylinder and add to the whirl-pak bag.
- (3) Securely close the whirl-pak bag and shake vigorously by hand or mechanical shaker (with similar hand shaking motion) for 3 minutes.
- (4) Allow the sample to settle for 1 minute. Then filter 3 mL of the extract with a filter syringe (Neogen item #9420) into a clean sample collection tube labeled with the sample identification.
- (5) For corn gluten meal, 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 to dilution procedure.
- (6) Using a 500  $\mu$ L pipettor, dilute the filtered sample 1:1 by adding 500  $\mu$ L of the filtered sample to 500  $\mu$ L of extraction solvent in a new test tube. Vortex for 10 seconds. This **diluted filtered extract** is ready for testing.
  - (7) Proceed to Test Procedures section.

#### b. Extraction Procedures for: Distillers Dried Grains with Solubles (DDGS).

- (1) Transfer 50 g  $\pm$  0.2 of ground sample into an extraction mixing jar.
- (2) Measure 150 mL of extraction solvent using a 250 mL graduated cylinder and add to the whirl-pak bag.
- (3) Securely close the whirl-pak bag and shake vigorously by hand or mechanical shaker (with similar hand shaking motion) for 3 minutes.

- (4) Allow the sample to settle for 1 minute. Then filter 3 mL of the extract with a filter syringe (Neogen item #9420) into a clean sample collection tube labeled with the sample identification.
- (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 to dilution procedure.
- (6) Using a 500 µL pipettor, dilute the filtered sample 1:1 by adding 500 µL of the filtered sample to 500 µL of extraction solvent in a new test tube. Vortex for 10 seconds. This **diluted filtered extract** is ready for testing.
- (7) Proceed to **Test Procedures** section.

#### 4. TEST PROCEDURES

**NOTE: For all unknown samples, analysis procedures A should be analyzed first. If the result is above 100 ppb, proceed to analysis procedure B.**

##### a. Analysis Procedure (5.0 – 100 ppb 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 pipettor with a new pipette tip, add 500 microliters (µL) of sample diluent to each red sample dilution cup.
- (3) Using a 100 µL pipettor add 100 µL of sample 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.
- (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 Aflatoxin 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 minute incubation/development period, remove the test strip from the sample cup. Read the test strip within one minute using only Neogen's Reveal AccuScan Gold Reader.

**b. Analysis Procedure (100 – 300 ppb Quantitation Range)**

- (1) Using the **diluted filtered extract** made in the Standard Extraction Procedure above, dilute the diluted filtered extract three-fold as described in step (2) below with 65% ethanol to prepare **Diluted Extract A**.
- (2) Measure 200 µL of 65% ethanol using a 500 µL pipettor into a new test tube. Using a 100 µL pipettor add 100 µL of diluted filtered extract to the ethanol. This is Diluted Extract A. Vortex for a few seconds prior to the analysis.
- (3) Follow the same test procedure as described in "a. Analysis Procedure (5.0 – 100 ppb Quantitation Range)" except instead of 100 µL of the diluted filtered extract use 100 µL of Diluted Extract A.
- (4) The result of this Diluted Extract A must be multiplied by 3 to obtain the final Aflatoxin concentration of the original test sample. Results using this protocol are valid in the range of 100- 300 ppb.

**c. 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) 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 inserted the Reveal Q+ test strip into the black cartridge adapter with the sample end first and results facing out.
- (4) Insert the cartridge with test strip side up into the AccuScan.
- (5) The reader will automatically begin analyzing the cartridge.

## **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@ams.uds.gov](mailto:Patrick.J.McCluskey@ams.uds.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) Test strip development times, other than those specified in Test Procedures section, may give inaccurate results.
- (3) The test strips must remain inside the stay-dry tube before use.
- (4) Treat all used liquids, including sample extract, and labware as if contaminated with Aflatoxin, 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.
- (6) Ethanol is highly flammable. Keep container tightly closed and away from heat, sparks, open flame and those who are smoking. It is toxic if swallowed, or if vapor is inhaled. Avoid contact with skin.
- (7) 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 Aflatoxin test strips, 25 red sample dilution cups
- (2) 25 clear sample cups, 1 bottle of sample diluent

### b. Materials required but not provided.

- (1) Timer (Neogen item #9426),
- (2) 65% ethanol solution (Neogen item #8071, #8072)
- (3) 100 µL pipettor (Neogen item #9272, #9278), 100 µL pipette tips (Neogen item #9407, #9410, #9417)

- (4) 500 µL pipettor (Neogen item #9291, #9336), 200-1000 µL pipette tips (Neogen item #9464, #9487, #9292, #9293)
- (5) Sample collection cups with lids. (Neogen item #9428),
- (6) Reveal sample rack (Neogen item #9475)
- (7) Reveal AccuScan Gold Reader (Neogen item #9595)
- (8) Disposable polyethylene transfer pipettes
- (9) Dispensing pump or graduated cylinder (Neogen item #9448, #9447), Filter Syringe (Neogen item #9420)
- (10) Agri-Grind grinder or equivalent (Neogen item #9427)
- (11) Scale capable of weighing 5 – 50 grams (Neogen item #9427)
- (12) Sample collection tubes with caps (Neogen item #9421, #9421B)
- (13) 10 mL, 250 mL, 500 mL and 1000 mL graduated cylinders

## **8. REVISION HISTORY**

8085 Effective 05/29/2018