

# Test Kit Instructions

December 9, 2019

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## **NEOGEN CORPORATION**

### **REVEAL Q+ MAX FOR AFLATOXIN QUANTITATIVE AFLATOXIN**

#### **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 (TSD) by phone at 816-891-0417 or email at [Ajit.K.Ghosh@usda.gov](mailto:Ajit.K.Ghosh@usda.gov).

Refer to the Mycotoxin Handbook for information on use of this test kit in official FGIS 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 the Policies, Procedures, and Market Analysis Branch (PPMAB) by phone at 816-659-8403 or email at [Patrick.J.McCluskey@usda.gov](mailto:Patrick.J.McCluskey@usda.gov).

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## 1. GENERAL INFORMATION

The REVEAL Q+ MAX 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 distilled or deionized water and a MAX 1 extraction packet along with an Aflatoxin-antibody particle complex coated test strip. The test strips are analyzed on the Neogen AccuScan Gold reader.

Approved Test Kit Information	
<b>Test Kit Vendor:</b>	Neogen Corporation 800/234-5333
<b>Test Kit Name:</b>	REVEAL Q+ MAX FOR AFLATOXIN
<b>Product Number:</b>	8088
<b>Effective Date of Instructions:</b>	12/09/2019
<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), pearl or pearled barley (including quick pearl barely), popcorn, brown rice, sorghum, soybeans, and wheat (including whole grain wheat flour, wheat middlings, wheat red dog, wheat flour 2nd clear, and wheat screenings)
<b>Extraction method:</b>	Blend 50g sample and one packet of MAX-1 G50 extraction powder in 250 mL (for soybeans use 350 mL) of distilled or deionized water for 30 seconds.
<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) Select Mycotoxin Q+ MAX for the Category.
- (6) Select the Q+ M Afla test type. See Analysis Procedure to determine which one to use.

## **3. SAMPLE PREPARATION AND EXTRACTION PROCEDURES**

Corn (including dent or field corn, corn meal, corn flour, cracked corn, corn grits or polenta, and corn screenings), pear or pearled barley (including quick pearl barley), popcorn, brown rice, sorghum, soybeans, and wheat (including whole grain wheat flour, wheat middlings, wheat red dog, wheat flour 2nd clear, and wheat screenings).

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

### **b. Sample extraction**

- (1) Weigh  $50.0 \pm 0.2$  grams of ground sample into a blender jar.
- (2) Add one packet of MAX 1-G50 Aqueous Extraction Powder to the jar. (CAUTION: Do not inhale the powder. Perform this step in a vent hood if possible.
- (3) Add 250 mL (350 mL for soybeans) of distilled or deionized water using a 250 mL graduated cylinder.
- (4) Blend for 30 seconds.

- (5) Pipette (using a transfer pipet) the sample extract into a 2 mL mini centrifuge tube and centrifuge for 30 seconds.
- (6) The supernatant of the centrifuged sample extract should be free of particulates and ready for testing or dilution as applicable.
- (7) Proceed to Test Procedures below.

#### **4. TEST PROCEDURES**

- a. AccuScan Gold Analysis Procedure (for quantitation range 5-50 ppb).
  - (1) Set up one red sample dilution cup and one clear sample cup for each extract to be tested. Place the cups in a holder or rack, labeled as applicable. Follow the same procedure for each extract.
  - (2) Using 100  $\mu$ L pipette, add 100  $\mu$ L of sample diluent to the red sample dilution cup.
  - (3) Using 100  $\mu$ L pipette, add 100  $\mu$ L of supernatant of the centrifuged sample extract to the red cup containing the diluent and mix by pipetting the mixture up and down at least 5 times.
  - (4) Pipette 100  $\mu$ L of the contents of the red cup into a new clear cup.
  - (5) Place a new Reveal Q+ MAX for Aflatoxin test strip into the clear cup, with the arrow end down, and develop for 6 minutes.
  - (6) Remove strip from the sample cup as soon as the 6-minute development is complete and read the results within 30 seconds.
  - (7) Fully insert the Reveal Q+ test strip into the black cartridge adapter, sample end first and results facing out.
  - (8) Insert the cartridge with test strip side up into the AccuScan reader. The reader will automatically read the strip and display the result.
    - (a) If the centrifuged sample extract was undiluted, the result displayed on the reader is the final result for the sample.
    - (b) If the result (diluted or undiluted) is above 50 ppb, the sample must be re-analyzed after appropriate dilution to bring it down to a level between 5 and 50 ppb.

- b. Notes about reading the results.
- (1) The strips must be read immediately after development using Neogen's AccuScan Gold Reader. Test results will be displayed and stored in the reader.
  - (2) Readings should be made between 6 minutes and 6 minutes and 30 seconds. Reading results after 6 minutes and 30 seconds may be inaccurate due to over-development of the test strip and should not be reported.
- c. Dilution A Protocol (for quantitation range 50-300 ppb).
- (1) Make a high-level diluent by adding one packet of MAX 1-G50 Aqueous Extraction Powder to 100 mL of distilled water and shaking by hand or a mechanical shaker for 3 minutes.
  - (2) Filter the diluent through a Neogen syringe filter.
  - (3) Dilute the **supernatant centrifuged sample extract** by 6 fold as follows:
    - (a) Using a 1000 µL pipette, add 500 µL of the **high-level diluent** to a test tube.
    - (b) Using a 100 µL pipette, add 100 µL of the **supernatant centrifuged sample extract** to the test tube and vortex for 10 seconds.
    - (c) This is the **Diluted Extract A**.
  - (4) Follow the same procedure as described in “a. AccuScan Gold Analysis Procedure (for quantitation range 5 – 50 ppb)” except in place of 100 µL **supernatant centrifuged sample extract** use 100 µL **Diluted Extract A**.
  - (5) To obtain the final Aflatoxin concentration you must multiply the result of this **Diluted Extract A** by Six. Results following this protocol are valid in the range of 50 – 300 ppb.

## 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@usda.gov](mailto:Patrick.J.McCluskey@usda.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 new tips for each measurement.
- (6) 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
- (2) 25 red sample dilution cups
- (3) 25 clear sample cups
- (4) 1 bottle of sample diluent

- (5) 25 MAX Aqueous Extraction packets
- (6) Instructions for use
- b. Materials required but not provided.
  - (1) Extraction Materials
    - (a) Sample collection cups with lids 125 mL (Neogen item #9428, #9428B)
    - (b) Sample collection tubes with caps (Neogen item #9421, 9421B)
    - (c) Neogen filter syringe, Whatman #1 filter paper, or equivalent (Neogen item #9420, 9519, 9429)
    - (d) Centrifuge, mini (Neogen item #9330)
    - (e) Micro centrifuge tubes (Neogen item #9172)
    - (f) Dispensing pump or graduated cylinder (Neogen item #9448, #9447)
  - (2) Timer (Neogen item #9426)
  - (3) 100 µL pipettor (Neogen item #9272, #9278)
  - (4) 100 µL pipette tips:
    - (a) Pipette tip rack (Neogen item #9407)
    - (b) Bag of 1000 (Neogen item #9410)
    - (c) Reload decks, 10 decks (Neogen item #9417)
  - (5) 500 µL pipettor (Neogen item #9291, #9336)
  - (6) 1000 µL pipettor (Neogen item #9463)
  - (7) 200-1000 µL pipette tips
    - (a) Pipette tip rack (Neogen item #9487)
    - (b) Bag of 1000 (Neogen item #9464, 9293)



- (c) Reload decks, 5 decks (Neogen item #9292)
- (8) Reveal sample rack (Neogen item #9475)
- (9) Reveal AccuScan Gold Reader (Neogen item #9595)
- (10) Disposable polyethylene transfer pipettes (Fisher Scientific Cat No. 13-711-7M)
- (11) Agri-Grind grinder or equivalent (Neogen item #9427)
- (12) Scale capable of weighing 5 – 50 grams (Neogen item #9427)
- (13) Distilled or deionized water
- (14) MAX-1 G50 Aqueous Extraction packets (Neogen item #8089G)
- (15) 250 mL (Neogen item #9368) and 500 mL (Neogen item #9345) graduated cylinders

## **8. REVISION HISTORY**

Effective 12/09/2019

- Soybeans was approved and added as an additional commodity.

Effective 07/12/2019