

Test Kit Instruction

December 12, 2018

NEOGEN REVEAL Q+ FOR AFLATOXIN USING RAPTOR INTEGRATED ANALYSIS PLATFORM

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.

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 PPMAB by phone at 816-659-8403 or email at Patrick.J.McCluskey@ams.usda.gov.

The U.S. Department of Agriculture (USDA) prohibits discrimination in its programs on the basis of race, color, national origin, sex, religion, age, disability, political beliefs, and marital or familial status. (Not all prohibited bases apply to all programs.) Persons with disabilities who require alternate means for communication of program information (Braille, large print, audiotape, etc.) should contact USDA's TARGET Center at (202) 720-2600 (voice and TDD).

To file a complaint, write to the USDA, Office of Civil Rights, Room 326-W, 1400 Independence Avenue, SW, Washington, DC 20250-9410, or call (202) 720-5964 (voice and TDD). USDA is an equal employment opportunity employer.

Contents

FORWARD 1

1. GENERAL INFORMATION..... 3

2. PREPARATION OF TESTING MATERIALS..... 4

3. SAMPLE PREPARATION AND EXTRACTION PROCEDURES..... 6

4. TEST PROCEDURES 7

5. REPORTING AND CERTIFYING TEST RESULTS..... 8

6. STORAGE CONDITIONS AND PRECAUTIONS 9

7. EQUIPMENT AND SUPPLIES 10

8. REVISION HISTORY..... 10

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 Raptor Integrated Analysis Platform (Raptor).

| Approved Test Kit Information | |
|---|---|
| Test Kit Vendor: | Neogen Corporation 800/234-5333 |
| Test Kit Name: | Reveal Q+ for Aflatoxin |
| Product Number: | 8085 |
| Effective Date of Instructions: | 11/27/2018 |
| Conformance Range: | 5.0 – 300 ppb |
| Number of Analyses to Cover Conformance Range: | 2 |
| Type of Service: | Quantitative |
| Supplemental Analysis: | Yes |
| Approved Commodities: | Corn (including dent or field corn, corn meal, corn flour, cracked corn, corn grits or polenta, and corn screenings), corn germ meal, corn gluten meal, corn/soy blend, corn starch, distillers dried grain with solubles (DDGS), popcorn, rough rice, sorghum, and wheat (including whole grain wheat flour, wheat middlings, wheat red dog, wheat flour 2nd clear, and wheat screenings). |
| Extraction method: | Vigorously shake 50 gram sample with 125 mL of 65% ethanol/35% distilled or deionized water (v/v) for 3 minutes. |
| Test Format: | Lateral flow strip |
| Detection Method: | Raptor Integrated Analysis Platform, Model #9680 |

2. PREPARATION OF TESTING MATERIALS

a. Raptor Integrated Analysis Platform 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) Fully insert a Reveal Q+ Aflatoxin test strip into a Raptor cartridge
- (2) Insert the Raptor cartridge containing the test strip into any of the three ports within the Raptor.
- (3) The bar code on the test strip will be read. The system identifies the type of test strip and the lot number. If the lot number is not found in the system, the bar code reader in the front of the Raptor will turn on automatically.
- (4) Scan the QR code found on the tube containing the test strips. The information will be stored on the reader.
- (5) Enter Sample ID if desired.

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).**

Note: If not using Neogen's premade extraction solvent, one can prepare a 65% ethanol/35% deionized or distilled water solution (v/v) by mixing 6.5 parts ACS grade ethanol (e.g. Fisher Scientific catalog#A407) with 3.5 parts distilled or deionized water as described below.

- (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).

Standard Extraction Procedure

- a. Transfer 50 g (\pm 0.2) of ground sample into the whirl-pak bag.
- b. Measure 125 mL of extraction solvent using a 250 mL graduated cylinder and add to the whirl-pak bag (for DDGS add 150 ml of extraction solvent).
- c. Securely tie the whirl-pak bag and shake vigorously by mechanical shaker (250 rpm) for 3 minutes or by hand shaking (with similar shaking motion) for 3 minutes.
- d. 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. Filtered extract is good for up to 4 hours if capped to avoid the evaporation.
- e. For DDGS, 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.

- (1) 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.
 - (2) 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.
- f. Dilute the filtered sample 1:1 by adding 0.5 mL of the filtered sample to 0.5 mL of extraction solvent in a new test tube. Vortex for 10 seconds. This **diluted filtered extract** is ready for testing and good for up to 4 hours if capped to avoid evaporation.
 - g. Proceed to **Test Procedures** section.

4. TEST PROCEDURES

NOTE: For all unknown samples, analysis procedures “5.0 – 100 ppb Quantitation Range” should be analyzed first. If the result is above 100 ppb, proceed to “100 – 300 ppb Quantitation Range” analysis procedure.

a. Analysis Procedure (5.0 – 100 ppb Quantitation Range)

- (1) Place the appropriate number of red sample dilution cups for each test sample in the sample cup rack. Label cups if necessary.
- (2) Using a single-channel 1000 µL 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 **diluted filtered extract** into each red dilution cup with sample diluent. Mix by swirling with the pipette tip and then by pipetting up and down 5 times. This is **Diluted Extract A**.
- (4) Fully insert a Reveal Q+ Aflatoxin test strip into a Raptor cartridge.
- (5) Insert the Raptor cartridge containing the test strip into any of the three ports within the Raptor.
 - (a) The bar code on the test strip will be read. The system identifies the type of test strip and the lot number. If the lot number is not found in the system, the bar code reader in the front of the Raptor will turn on automatically.
 - (b) Scan the QR code found on the tune containing the test strips. The information will be stored on the reader.
- (6) Add 400 µL (using an adjustable 100 – 1000 µL pipette) of the **Diluted Extract A** from the red sample dilution cup to the Raptor cartridge.
 - (a) The Raptor system will start automatically or press “continue” to move to the “detect fluid front” screen. Results will be displayed upon the completion of the 6 minutes incubation.
 - (b) Additional samples can be started in the other ports while the first sample is processing.
- (7) Results following this protocol are valid in the range of 5.0 – 100 ppb. If the result is more than 100 ppb, run the test using extraction procedure for 100 –300 ppb quantitation range below.

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

- (1) Dilute the **diluted filtered extract** (from #6 of Standard Extraction Procedure) three-fold with 65% ethanol as described below.
- (2) Using a 100 µL pipettor, add 100 µL of **diluted filtered extract** into a clean test tube. Using a 1000 µL pipettor, add 200 µL of 65% ethanol into the test tube containing **diluted filtered extract**. Vortex for a few seconds. This is **Diluted Extract B**.
- (3) Using a single-channel 1000 µL pipettor, add 500 microliters (µL) of sample diluent to a red sample dilution cup.
- (4) Using a 100 µL pipettor, add 100 µL of **Diluted Extract B** into the red dilution cup containing sample diluent. Mix by swirling with the pipette tip and then by pipetting up and down 5 times. This is **Diluted Extract C**.
- (5) Follow the rest of the test procedure as described in “**A. Analysis Procedure (5.0 – 100 ppb Quantitation Range)**.”
- (6) The result at the screen must be multiplied by 3 to obtain the final aflatoxins concentration in the original sample. Results using this protocol are valid in the range of 100- 300 ppb. If the final result is less than 53 ppb, perform assay with another test strip using 5.0 – 100 ppb

Quantitation Range. If the result is still more than 100 ppb, perform assay with another test strip using 100 – 300 ppb Quantitation Range.

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).

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) 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.
- (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, 25 red sample dilution cups
- (2) 1 bottle of sample diluent

b. Materials required but not provided.

- (1) Timer (Neogen item #9426),
- (2) Neogen's Premade 65% ethanol solution (Neogen item #8071, #8072), ACS grade ethanol Fisher Scientific catalog#A407
- (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) Raptor Integrated Analysis Platform (Neogen item #9680)
- (8) Raptor Cartridges (Neogen item #9681)
- (9) Disposable polyethylene transfer pipettes
- (10) Dispensing pump or graduated cylinder (Neogen item #9448, #9447), Filter Syringe (Neogen item #9420)
- (11) Agri-Grind grinder or equivalent (Neogen item #9427)
- (12) Scale capable of weighing 5 – 50 grams (Neogen item #9427)
- (13) Sample collection tubes with caps (Neogen item #9421, #9421B)
- (14) 10 mL, 250 mL, 500 mL and 1000 mL graduated cylinders

8. REVISION HISTORY

Effective 12/12/2018