

Test Kit Instruction

July 7, 2015

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@usda.gov.

Refer to the Mycotoxin Handbook 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@udsa.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. EXTRACTION PROCEDURES.....	6
4. TEST PROCEDURES	7
5. SUPPLEMENTAL ANALYSIS	8
6. REPORTING AND CERTIFYING TEST RESULTS.....	8
7. STORAGE CONDITIONS AND PRECAUTIONS.....	9
8. EQUIPMENT AND SUPPLIES	10
9. 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 AccuScan Gold reader.

Approved Test Kit Information	
Test Kit Vendor:	<i>Neogen Corporation 800/234-5333</i>
Test Kit Name:	Reveal Q+ for Aflatoxin
Product Number:	8085
Effective Date of Instructions:	07/07/2015
Instructions Revision Number:	0
Conformance Range:	5 – 100 ppb
Number of Analyses to Cover Conformance Range:	1
Type of Service:	Quantitative
Supplemental Analysis:	Yes
Approved Commodities:	Corn, brewer's rice, corn flaking grits, corn germ meal, corn gluten meal, corn meal, corn screenings, corn starch, corn/soy blend, cottonseed, cracked corn, distillers dried grains with solubles (DDGS), popcorn, rough rice, sorghum, and wheat Extraction method:
Extraction method:	Vigorously shake 50 gram sample with 125 mL of 65% ethanol/35% distilled or deionized water for 3 minutes.
Test Format:	Lateral flow strip
Detection Method:	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.

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 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) Add slowly 8.2 mL of 12.1N HCl (concentrated Hydrochloric acid) into 91.8 mL 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. EXTRACTION PROCEDURES

a Extraction Procedures for: corn, cracked corn, corn flaking grits, corn germ meal, corn gluten meal, corn meal, corn screenings, corn starch, corn/soy blend, popcorn, brewers rice, sorghum, rough rice and wheat.

- (1) Transfer 50 g (\pm 0.2) of ground sample into the whirl-pak bag.
- (2) Add 125 mL of extraction solvent.
- (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 – 5 mL of the extract with a filter syringe (Neogen item #9420) into a clean sample collection tube labeled with the sample identification.
- (5) Diluter 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.
- (6) 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) Add 150 mL of extraction solvent.
- (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 – 5 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) Proceed to Test Procedures section.

4. TEST PROCEDURES

a. Analysis Procedure.

- (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 new pipette tip, 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) 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. Reading the Results.

- (1) The strips must be immediately 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 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. SUPPLEMENTAL ANALYSIS

Supplemental analysis (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 aflatoxin test kits is 5 – 100 ppb. Therefore, supplemental analysis would be performed for a result above 100 ppb. 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., 5 -100 ppb for Aflatoxin), and a correction for dilution is applied to derive at the final result. Supplemental analysis is performed only at the request of the applicant.

Supplemental Dilution Procedure.

- (a) Combine 100 µL of diluted filtered extract (from extraction procedure above) with 100 µL of extraction solvent.
- (b) Mix by swirling or pipetting up and down 5 times. This is the supplemental diluted filtered extract for supplemental analysis.
- (c) Refer to Test Procedures section for analysis.
- (d) Read and record results on the work record, then multiply the analytical results obtained from the AccuScan reader by 2 to obtain the actual aflatoxin concentration of the original test sample (show all results on the work record).

Example: If the filtered extract is diluted 1:1 (v/v) using extractions solvent, the dilution factor is two (2).

AccuScan Gold reader supplemental analysis results:	90 ppb
<u>Multiplied by the dilution factor</u>	<u>x 2</u>
Sample results - TOTAL :	180 ppb

A final result less than 53 ppb is indicative of a problem, and troubleshooting is needed. Verify the procedure is being followed properly. Perform the procedure for the Diluted Extract (non- supplemental analysis) and only perform the supplemental analysis again if the value is greater than 100 ppb.

6. 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).

7. 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.

8. 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), 65% ethanol solution (Neogen item #8071, #8072)
- (2) 100 μ L pipettor (Neogen item #9272, #9278), 100 μ L pipette tips (Neogen item #9407, #9410, #9417)
- (3) 500 μ L pipettor (Neogen item #9291, #9336), 200-1000 μ L pipette tips (Neogen item #9464, #9487, #9292, #9293)
- (4) Sample collection cups with lids. (Neogen item #9428), Reveal sample rack (Neogen item #9475)
- (5) Reveal AccuScan Gold Reader (Neogen item #9595), Disposable polyethylene transfer pipettes
- (6) Dispensing pump or graduated cylinder (Neogen item #9448, #9447), Filter Syringe (Neogen item #9420)
- (7) Agri-Grind grinder or equivalent (Neogen item #9427), Scale capable of weighing 5 – 50 grams (Neogen item #9427), Sample collection tubes with caps (Neogen item #9421, #9421B)

9. REVISION HISTORY

Revision 0 (07/07/2015)