

Program Notice

FGIS-PN-12-03

6/11/12

REVEAL Q+ FOR AFLATOXIN TEST METHOD

1. PURPOSE

The purpose of this program notice is to provide instructions for the Reveal Q+ Aflatoxin test method, product number 8085, recently approved by the Technology and Science Division for official use.

2. BACKGROUND

The Grain Inspection, Packers, and Stockyards Administration's (GIPSA), Field Management Division (FMD), Policies, Procedures, and Market Analysis Branch (PPMAB), and the Technology and Science Division (TSD) evaluate new test methods, and improvements to previously approved methods, in order to provide the market with performance-verified rapid mycotoxin test kits.

In order to offer newly approved/revised test methods in a timely manner, this program notice is issued prior to the release of the revised Aflatoxin Handbook. The following test procedures are approved for official use by field offices and official service providers.

3. TEST PROCEDURES

a. 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 aflatoxin. Using an aflatoxin-antibody particle complex coated test strip and the AccuScan III reader, the inspector is able to determine the presence of aflatoxin in a sample by following approved test procedures and Neogen's assay principles.

The test kit is limited to providing quantitative aflatoxin measurements between **5 and 100 ppb**. **For reporting test results above 100 ppb a supplemental analysis must be performed or test results are reported as exceeding 100 ppb.** Refer to section f. (3) of the program notice for supplemental analysis procedures.

Obtain samples according to the instructions in the Grain Inspection Handbook, Book I “Grain Sampling,” and refer to the Aflatoxin Handbook Chapter 3 “Sample Preparation for minimum sample requirement and sample preparation procedures.”

The extraction solution and other materials used with this test method necessitate the use of a separate FGIS-approved laboratory space. Federal Grain Inspection Service (FGIS) employees must comply with all applicable safety and sanitation requirements as listed in the Aflatoxin Handbook, Chapter 2, Laboratory Safety to ensure a safe and efficient work environment.

Approved Test Kit Information					
Test Kit Vendor:	Neogen Corporation 1-800-234-5333				
Test Kit Name:	Reveal Q+ for Aflatoxin Test Method				
Product Number	Conformance Limits		Type of Service	Extraction Solution	Supplemental Analysis
8085	Min	Max	Quantitative	65% Ethanol (reagent grade or better)	Yes
QAC Number	5 ppb	100 ppb			
AFLGC					
Grain/Commodities Approved for					
corn, brewers rice, corn flaking grits, corn germ meal, corn gluten meal, corn meal, corn screenings, corn/soy blend, corn starch, cracked corn, distillers dried grains with solubles, popcorn, and sorghum.					

b. Preparation of Extraction Solution.

The extraction solvent used in the Reveal Q+ for Aflatoxin test method is an ethanol/water solution consisting of 65/35 percent ethanol-water (distilled or deionized).

(1) Ethanol/Water.

- (a) Using a graduated cylinder, measure 650 milliliters (ml) of ethanol and place it into a clean carboy with spigot.
- (b) Add 350 ml distilled or deionized water to the ethanol and shake vigorously until it is completely mixed.
- (c) Label the container stating the mixture (65/35 percent ethanol-water), date of preparation, and initials of the technician who prepared the solution.

- (d) Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of - 6.5 parts ethanol to 3.5 parts distilled or deionized water.

c. Reveal AccuScan III Reader Set Up.

The AccuScan III reader must have software version 4.2 or newer to analyze the Reveal Q+ tests. Contact Neogen for more information.

- (1) Launch the AccuScan III program on the reader.
- (2) Select the Mycotoxin Q+ category and Q+ Aflatoxin test.
- (3) Enter a sample ID (optional).
- (4) Enter the test kits lot number, and A, B, C, and D values from the test it box label or select from the history for previously entered lot number details.

Note: Refer to the AccuScan III manual for more detailed instructions. The lot number in the AccuScan III reader must match the lot number of test strips being analyzed.

****Technicians must update or verify reader information (lot number of test strips in use) before official testing.****

d. Extraction Procedures.

- (1) Transfer 50 grams of ground sample into an extraction mixing jar.
- (2) Add 250 ml of 65/35 ethanol/water extraction solution.
- (3) Cover the extraction jar shake vigorously for 3 minutes.
- (4) Allow the sample to settle. Then filter 3 – 5 ml of the extract with a filter syringe into a clean sample collection tube labeled with the sample identification.

Note: The use of a filter syringe is the only TSD approved filtering procedure for this test kit. Do not use any paper filters for filtering.

- (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) Proceed to section f, "Test Procedures".
- e. Extraction Procedures for Distillers Dried Grains with Solubles (DDGS).
- (1) Transfer 50 grams of DDGS into an extraction jar.
 - (2) Add 150 ml of 65/35 percent ethanol/water extraction solution.
 - (3) Cover the extraction jar and shake vigorously for 3 minutes.
 - (4) Allow the sample to settle. Then filter 3 - 5 ml of the extract with a filter syringe into a clean sample collection tube labeled with the sample identification.
 - (5) After collecting the filtered extract, dispose of the filter syringe and ground material according to waste disposal guidelines. Set the filtrate aside for pH verification or adjustment if needed.
 - (6) Check the pH of the filtered extract using a pH strip. If pH is below 6.0 the sample need to be adjusted.

To Adjust pH:

- (a) Using a disposable polyethylene transfer pipette, add one drop of 1N NaOH (sodium hydroxide) to the sample extract, vortex or swirl to mix, and check the pH with pH test strip.
 - (b) If pH is still below 6.0, add another drop of 1N NaOH, mix, and check pH again. Continue this process until the pH falls between 6.0 an 8.0.
 - (c) Proceed to section f, "Test Procedures".
- f. Test Procedures.
- (1) Sample Analysis.
 - (a) 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.

- (b) Using a single-channel pipettor with a new pipette tip, add 500 microliters (μ l) of sample diluent to each red sample dilution cup.
- (c) Using a new pipette tip, add 100 μ l of sample extract into each red dilution cup with sample diluents. Mix by pipetting up and down 5 times, discard tip.
- (d) Transfer 100 μ l of diluted sample extract into a new clear sample cup.
- (e) Place a new Reveal Q+ for Aflatoxin test strip with the sample end down into the sample cup. Set timer and incubate for 6 minutes.
- (f) At the end of the 6 minute incubation/development period, remove the test strip from the sample cup. Proceed directly to reading test results.

(2) Reading Test Results with the AccuScan III Reader.

Test strips should be read within 1 minute after completion of the 6 minute incubation period. Refer to Reveal AccuScan III manual for detailed set-up and selection information.

- (a) Fully insert the Reveal Q+ test strip into the black cartridge adapter with the sample end first and results facing out.
- (b) Insert the cartridge with test strip upside-down into the reader (the test lines will face downward into the reader).
- (c) The reader's green light will glow when a cartridge is inserted, and will automatically begin analyzing the cartridge.
- (d) The AccuScan III reader will analyze the test strip. Test results will be displayed, and stored in the 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.

- (2) Sample results over 100 ppb are reported as greater than 100 ppb (> 100 ppb) unless a supplemental analysis is performed.
- (3) Refer to the Aflatoxin Handbook, Chapter 4, Certification, for more detailed certification statements and procedures.

h. Cleaning Labware.

(1) Negative Tests (≤ 20 ppb).

(a) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used extraction mixing jars, wash thoroughly, and then rinse with clean water before reusing.

(b) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

(2) Positive Tests (> 20 ppb).

(a) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used extraction mixing jars and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, and then rinse with clean water before reusing.

(b) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution". Soak disposable materials, such as used test strips and pipettes for at least 5 minutes. Pour the liquid down the drain and then place the materials in a garbage bag for disposal.

i. Waste Disposal.

(1) Negative Results (≤ 20 ppb).

If the test result is negative “equal to or less than 20 ppb”, dispose of any remaining liquid filtrate in the chemical waste container. Discard the sample slurry (ground material) into a plastic garbage bag for disposal.

(2) Positive Results (> 20 ppb).

If the result is positive “more than 20 ppb”, the slurry (ground portion) remaining in the sample extraction jar must be decontaminated prior to disposal. After disposing of the remaining filtered extract in the chemical waste container, pour approximately 50 ml of bleach solution into the sample extraction jar, then shake to mix with the sample slurry. After the slurry and bleach solution separate, handle the bleach rinse filtrate as a non-hazardous solution. Dispose by pouring the liquid down the drain. Discard the sample slurry portion into a plastic garbage bag for disposal.

j. Equipment and Supplies.

(1) Materials Supplied in Test Kits.

- (a) 25 Reveal Q+ aflatoxin test strips.
- (b) 25 red sample dilution cups.
- (c) 25 clear sample cups.
- (d) 1 bottle of sample diluent.

(2) Materials, Supplies and Equipment Recommend but not Provided.

- (a) Timer. (Neogen item #9426)
- (b) 100 µl pipettor (or equivalent) with pipette tips.
- (c) 500 µl pipettor (or equivalent) with pipette tips.
- (d) Sample collection cups with lids. (Neogen item #9428)
- (e) Reveal sample rack. (Neogen item #9475)
- (f) Sample Collection tubes with caps. (Neogen item #9421, #9421B)
- (g) Reveal AccuScan III Reader. (Neogen item #9590)

- (h) 65 percent Ethanol, reagent grade or better. (Neogen item #8071, #8072).
 - (i) pH test strips. (Neogen item #9478)
 - (j) Sodium hydroxide/(1N NaOH).
 - (k) Disposable polyethylene transfer pipettes.
 - (l) Distilled or Deionized Water.
 - (m) Dispensing pump or graduated cylinder. (Neogen item #9448, #9447)
 - (n) Filter Syringe. (Neogen item #9420)
 - (o) Pipette tips, 200, & 1000 μ l.
 - (p) Sample grinder.
 - (q) Scale capable of weighing 5 – 50 grams.
- k. Storage Conditions.
- (1) Storage Conditions.
 - (a) Store kit components at room temperature (18 - 30°C, 64°- 86°F) when not in use to ensure full shelf life.
 - (b) Test strips should remain capped in their original tubes until used to ensure optimal performance.
 - (c) Do not freeze test kit components.
 - (2) Precautions.
 - (a) Does not use test kit components beyond the expiration date.
 - (b) Test strip development times, other than those specified in Test Procedures, may give inaccurate results.
 - (c) Treat all used liquids, including sample extract, and labware as if contaminated with aflatoxins, gloves and other protective apparel should be worn at all times.

- (d) When using Sodium Hydroxide/1N NaOH gloves and other protective apparel should be worn. Avoid contact with any surface.
- (e) To avoid cross-contamination, use clean glassware for each sample, and thoroughly wash all glassware between samples.

4. FILING

Retain a copy of this program notice with the Aflatoxin Handbook until the handbook is revised to include the test procedures stated herein.

5. QUESTIONS

Direct any questions concerning this program notice to Carl Jackson, PPMAB, at (202) 720-8286, email carl.jackson@usda.gov or Patrick McCluskey, PPMAB, at (816) 659-8403, email patrick.j.mccluskey@usda.gov.

/s/ Robert Lijewski

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