

Program Notice

FGIS-PN-10-07

12/28/09

REVEAL® FOR AFLATOXIN

TEST METHOD

1. PURPOSE

The purpose of this program notice is to provide revised instructions for the Aflatoxin Handbook, Chapter 15, Reveal® for Aflatoxin Test Method, product number 8015, to include an optional ethanol extraction procedure.

2. BACKGROUND

The Grain Inspection, Packers, and Stockyards Administration (GIPSA), Field Management Division (FMD), Policies, Procedures, and Market Analysis Branch (PPMAB), and Technical Service Division (TSD)'s ongoing evaluation of new upgrades to previously approved test kits has impacted GIPSA's clearance process and licensing programs.

In an effort to enhance clearance procedures and offer TSD newly revised/approved 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 use by GIPSA and official service providers.

3. TEST PROCEDURES

a. General Information.

The Reveal® for Aflatoxin kit from Neogen is a single-step lateral flow immunochromatographic assay based on a competitive immunoassay format. The test provides a qualitative determination of the presence or absence of aflatoxin at a 20 parts per billion (ppb) threshold level. Using an aflatoxin-antibody particle complex coated test strip, the inspector is able to determine the presence of aflatoxin in the sample by the formation of a visible line on the test strip.

The test kit is limited to providing aflatoxin measurements (**equal to or less than 20 ppb or exceeding 20 ppb**).

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 this handbook 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® for Aflatoxin			
Product Number	Conformance Limits	Type of Service	Extraction Solution	Supplemental Analysis
	Threshold			
8015	Equal to or less than 20 ppb	Qualitative	Methanol or Ethanol (reagent grade or better)	No
Grain/Commodities Approved for				
Corn only				

b. Preparation of Extraction Solution.

The extraction solvent used in the Reveal® for Aflatoxin test method is either a methanol/water solution consisting of 70 percent methanol/30 percent water (distilled or deionized); or a 50 percent ethanol/50 percent water (distilled or deionized).

(1) Methanol/Water.

- (a) Using a graduated cylinder, measure 700 milliliters (ml) of methanol and place it into a clean carboy with spigot.
- (b) Add 300 ml distilled or deionized water to the methanol and shake vigorously until it is completely mixed.
- (c) Label the container stating the mixture (70 percent methanol and 30 percent 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 seven parts methanol to three parts of distilled or deionized water.

(2) Ethanol/Water.

- (a) Using a graduated cylinder, measure 500 ml of ethanol and place it into a clean carboy with spigot.
- (b) Add 500 ml distilled or deionized water to the ethanol and shake vigorously until it is completely mixed.
- (c) Label the container stating the mixture (50 percent ethanol and 50 percent 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 one part ethanol to one part distilled or deionized water.

c. Extraction Procedures.

- (1) Transfer 50 grams of ground sample into an extraction mixing jar.
- (2) Add 100 ml of the (70/30) methanol/water; or 100 ml of 50/50 ethanol/water extraction solution.
- (3) Cover the extraction jar and blend on high speed for 1 minute.
- (4) Allow the sample to settle, filter the extract through a filter syringe or filter paper, and place into a sample jar labeled with the sample identification.
- (5) After collecting the filtrate, dispose of the filter and ground material. Set the filtrate aside for sample analysis.

d. Test Procedures.

- (1) Sample Analysis.
 - (a) Remove the appropriate number of sample cups and place in a sample cup rack.
 - (b) Using a single or multichannel pipettor with a new pipette tip, add 200 microliters (μl) of sample diluent to a sample cup.
 - (c) Using a new pipette tip, add 200 μl of the clear portion of the filtered sample extract to the sample cup. (This is a 1:1 dilution.)
 - (d) Mix the solution by pipetting up and down three times.

- (e) Place a new aflatoxin test strip with the sample end down into the sample cup.
- (f) Allow the test strip to develop in the sample cup for 3 minutes.
- (g) At the end of the 3 minute development period, remove the test strip from the sample cup.
- (h) Read the results visually against a white background or with Neogen's Reveal AccuScan III Reader.

(2) Interpreting the Lateral Flow Test Strip by Visual Analysis.

Development of a line in the Control Zone of the test strip within 3 minutes indicates that the strip has functioned properly. Any strip that does not develop a line in the Control Zone should be discarded. A second preparation of the extract (using a fresh 1:1 dilution) should be made and tested using another test strip.

- (a) Negative Result (≤ 20 ppb).

If a line forms in the Test Zone and another line forms in the control zone within 3 minutes (resulting in two visible lines on the test strip), the sample is considered negative (equal to or less than 20 ppb) for aflatoxin. A negative sample may be determined before the full 3 minute development if two lines are visible on the strip.

- (b) Positive Result (> 20 ppb).

If after the full 3 minutes there is no visible line in the Test Zone, but a visible line exists in the Control Zone. The sample is considered positive (exceeding 20 ppb) for aflatoxin.

(3) Reading Lateral Flow Test Strip Results with the Reveal AccuScan III Reader.

The strips can be read using Neogen's Reveal AccuScan III reader that incorporates a PDA with a Reveal AccuScan III lateral flow optical reader. Using the reader helps eliminate subjectivity by interpreting, and storing sample results. Operating instructions are included with the Reveal AccuScan III Reader (optional equipment).

- e. Reporting and Certifying Test Results.

- (1) Report results on the pan ticket and inspection log as being equal to or less than 20 ppb (≤ 20 ppb), or as exceeding 20 ppb (> 20 ppb), as applicable.
- (2) Certify results as being equal to or less than 20 ppb or exceeding 20 ppb, as applicable.
- (3) Refer to the Aflatoxin Handbook, Chapter 4, Certification, for more detailed certification statements and procedures.

f. 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.

g. 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 and dispose of by pouring the liquid down the drain. Discard the sample slurry portion into a plastic garbage bag for disposal.

h. Equipment and Supplies.

(1) Materials Supplied in Test Kits.

- (a) 25 aflatoxin test strips.
- (b) 25 sample cups.
- (c) One bottle of sample diluent.

(2) Materials Required but not Provided.

- (a) Timer (3 minute capacity).
- (b) 200 µl pipettor (or equivalent) with pipette tips.
- (c) Sample cup holder.
- (d) Balance.
- (e) Sample Grinder.
- (f) Methanol, reagent grade or better.
- (g) Ethanol, reagent grade or better.
- (h) Distilled or Deionized Water.
- (i) Blender with mixing jars.

- (j) Filter Paper or Coffee Filters.
- (3) Optional Equipment.
 - (a) Reveal AccuScan III Reader.
- i. Storage Conditions.
 - (1) Storage Conditions.
 - (a) Store kit components at room temperature (64°- 86°F) when not in use to assure full shelf life.
 - (b) Test strips must remain capped in the stay-dry tube before use to assure optimal performance.
 - (c) Do not freeze test kit components.
 - (2) Precautions.
 - (a) Do not use test kit components beyond expiration date.
 - (b) Test strip development times other than those specified may give inaccurate results.

4. FILING

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

5. QUESTIONS

Direct any questions concerning this program notice to Carl Jackson, PPMAB, at (202) 720-8286, or Robert Lijewski, PPMAB, at (202) 720-0224.

/s/ Robert Lijewski

Robert Lijewski
Acting Director
Field Management Division