

NEOGEN
REVEAL FOR AFLATOXIN

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GENERAL INFORMATION

Reveal for Aflatoxin is a single-step lateral flow immunochromatographic assay based on a competitive immunoassay format. The extract is wicked through a reagent zone, which contains antibodies specific for aflatoxin conjugated to colored particles. If aflatoxin is present, it will be captured by the particle-antibody complex. The aflatoxin-antibody-particle complex is then wicked onto a membrane, which contains a zone of aflatoxin conjugated to a protein carrier. This zone captures any uncomplexed aflatoxin antibody, allowing the particles to concentrate and form a visible line. If aflatoxin is present above a pre-determined limit, no line will form. Therefore, a positive sample will result in the absence of a line in the test zone. Alternatively, a negative sample will form a visible line in the test zone. The membrane also contains a control zone where an immune complex present in the reagent zone is captured by an antibody, forming a visible line. The control line will always form regardless of the presence of aflatoxin, ensuring the strip is functioning properly.

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 current policies and/or instructions issued by the Policies, Procedures, and Market Analysis Branch (PPMAB) of the Field Management Division 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@usda.gov.

Approved Test Kit Information

Test Kit Vendor:	<i>Neogen Corporation 800/234-5333</i>
Test Kit Name:	Reveal for Aflatoxin
Product Number:	8015
Effective Date of Instructions:	02/18/2015
Instructions Revision Number	0
Performance Limit:	20 ppb
Type of Service:	Qualitative
Approved Commodities:	Corn
Extraction method:	Shake 50 gram sample with 100 mL of 70% methanol/30% deionized water (v/v) or 100 mL of 50% ethanol/50% deionized water (v/v) for 1 minute using mechanical shaker at 250 rpm (or shake vigorously by hand with similar shaking action).
Test Format:	Lateral flow strip
Detection Method:	Visual

SAMPLE PREPERATION AND EXTRACTION PROCEDURES

Refer to the current policies and/or instructions issued by the Policies, Procedures, and Market Analysis Branch of the Field Management Division for information regarding sampling and general sample preparation (grinding, dividing, etc.). The sample to be tested should be collected according to accepted sampling technique.

Preparation of Extraction Solutions:

- (1) If not using Neogen's prepared solution, prepare a 70% methanol/30% deionized water (v/v) solution by mixing 7 parts ACS Grade methanol with 3 parts distilled or deionized water for each sample.
 - a. Using a 1000 mL graduated cylinder, measure 700 mL of methanol (ACS reagent grade or better) and place it into a glass bottle or carboy.
 - b. Using a 500 mL graduated cylinder, add 300 mL of deionized or distilled water to the methanol and shake until completely mixed.
 - c. Label the container stating the mixture 70 % methanol/30% percent water (v/v), date of preparation, and initials of technician that prepared the solution.
 - d. Store this solution at room temperature in a tightly closed container.
 - e. To prepare smaller or larger amounts of the solution use the ratio of 7 parts methanol to 3 parts of distilled or deionized water.
- (2) If using 50% ethanol, prepare the solution by mixing 1 part reagent grade ethanol with 1 part distilled or deionized water by following the steps above.
- (3) To prepare 1 liter of 50% ethanol, add 500 mL ACS Grade ethanol into 500 mL distilled or deionized water and shake until completely mixed.

Standard Extraction Procedure:

- (1) Weigh 50 ± 0.2 grams ground samples into a whirl pack bag.
- (2) Add 100 mL of 70% methanol/30% deionized water (v/v) or 100 mL of 50% ethanol/50% deionized water (v/v) and close the bag securely to prevent spillage.
- (3) Shake vigorously by mechanical shaker at 250 rpm for one minute (or shake vigorously by hand with similar shaking action for one minute).
- (4) Filter about 2 to 3 mL of the extract through a Neogen syringe filter (Item #9420). The filtered extract is now ready for testing.

TEST PROCEDURES

a. Analysis Procedure.

- (1) Remove the appropriate number of sample cups and place in sample cup rack.
- (2) Using a pipettor and new tip add 200 µL of sample diluent (provided with the test kit) to a sample cup.
- (3) Using a new tip add 200 µL of the filtered extract to the sample cup. Mix by pipetting up and down 3 times.
- (4) Place a new Aflatoxin test strip with the sample end down into the sample cup.
- (5) Allow the strip to develop in the sample cup for 3 minutes.
- (6) Remove the strip and read results immediately by visual observation.

b. Interpretation of the Results

- (1) Results less than 20 ppb: If a line forms in the test zone and another line forms in the control zone within 3 minutes (two lines total), the sample contains less than 20 ppb aflatoxin (negative sample). A negative sample may be determined as soon as 2 lines are visible on the strip. If there is no line in the control zone, the test is invalid and the sample should be re-tested with another strip.
- (2) Results greater than or equal to 20 ppb: if after the full 3 minutes there is no visible line in the test zone, but a visible line in the control zone, the sample contains at least 20 ppb aflatoxin (positive sample). If there is no line in the control zone, the test is invalid and the sample should be retested with another strip.
- (3) Observations should be made between 3 and 4 minutes. Observations after 4 minutes may be inaccurate due to over-development of the strip.

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).

STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions

Store kit components at room temperature (18-30°C, 64-86°F) to assure full shelf life. The strips should remain capped in their original sample tubes until used to assure optimal performance.

b. Precautions

- (1) Methanol and ethanol solutions are highly flammable. Keep container tightly closed, and keep away from heat, sparks, open flame and those smoking. It is toxic if swallowed, or if vapor is inhaled. Avoid contact with skin.
- (2) The test strips must remain inside the stay-dry tube before use.
- (3) Store test kit at room temperature (18-30°C, 64-86°F) when not in use, do not freeze test kits.
- (4) Do not use kit components beyond expiration date.
- (5) 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.
- (6) To avoid cross-contamination, use new pipette tip for each measurement.

EQUIPMENT AND SUPPLIES

a. Materials Provided in Test Kits.

- (1) 25 Aflatoxin test strips
- (2) 25 sample cups
- (3) 1 bottle of sample diluent
- (4) Directions for use

b. Materials required but not provided.

- (1) 70% methanol solution (Neogen item #8055) or 50% ethanol
- (2) 1000, 500 and 100 mL graduated cylinder
- (3) Scale capable of weighing 50 grams
- (4) 200 µL pipettor (Neogen item #9276)

- (5) Tips for 200 μ L pipettor
- (6) Timer (Neogen item #9426)
- (7) Distilled or deionized water
- (8) Sample collection cups with lids (Neogen item #9428)
- (9) Reveal sample cup rack (Neogen item #9475)

REVISION HISTORY

Revision 0 (02/18/2015)