

NEOGEN
AGRI-SCREEN FOR AFLATOXIN

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GENERAL INFORMATION

Agri-Screen for Aflatoxin is a competitive direct enzyme-linked immunosorbent assay (CD-ELISA) which allows the user to determine how the concentration of aflatoxin in a sample compares to the concentration of aflatoxin in a supplied control of 20 ppb. A methanol/water solution is used to extract any existing aflatoxin from a ground sample. Extracted aflatoxin in a filtrate is mixed with enzyme-labeled aflatoxin (conjugate). The mixed solution is transferred to antibody-coated wells, where free aflatoxin and conjugate compete for antibody binding sites. After a wash step, substrate is added. Color develops as a result of the presence of bound conjugate. Red stopping reagent is added and the color of the resulting solution is observed. Blue color indicates negative samples. Red color indicates strong positives.

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:	Agri-Screen for Aflatoxin
Product Number:	8010
Effective Date of Instructions:	02/18/2015
Instruction Revision No.	0
Detection Threshold:	20 ppb
Type of Service:	Qualitative
Approved Commodities:	Corn
Extraction method:	Shake 50 gram sample with 250 mL of 70% methanol/ 30% deionized water (v/v) for 3 minutes using mechanical shaker at 250 rpm (or shake vigorously by hand with similar shaking action for 3 minutes).
Test Format:	Competitive direct enzyme-linked immunosorbent assay
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 solvent

- (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 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, measure 300 mL of deionized or distilled water and add to the 700 mL of methanol and shake until completely mixed.
 - c. Label the container stating the mixture (70 percent methanol : 30 percent water), 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.

Extraction Procedure for corn

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

TEST PROCEDURES

a. Analysis Procedure.

- (1) Allow reagents and antibody wells to reach room temperature (64 - 86° F) prior to running the test.
- (2) Remove 1 red-marked mixing well from the foil pack for each sample to be tested, and one for the control, and place in the well holder.

NOTE: Do not run more than 4 wells at a time unless you are using a multichannel pipettor. Contact Neogen for more information

- (3) Remove an equal number of antibody-coated wells. Return well which will not be used immediately back to the foil pack and reseal to protect the antibody. Mark one end of the strip with a "1", and place in the well holder with the marked end on the left.
- (4) Mix each reagent by swirling the reagent bottle prior to use.
- (5) Using a new pipette tip, add 100 µL of conjugate from the blue-labeled bottle to each red-marked mixing well. Discard the tip.
- (6) Using a new tip, add 100 µL of the control to the first red-marked well.
- (7) Thoroughly mix by inserting the tip in the liquid and pipetting up and down 5 times. Discard the tip.
- (8) Using a new tip, add 100 µL of the first sample to the second red-marked well.
- (9) Thoroughly mix by inserting the tip in the liquid and pipetting up and down 5 times. Discard the tip.
- (10) Repeat the process for each additional sample in a following red-marked well.
- (11) Using a new tip for each, transfer 100 µL from each red-marked well to the corresponding antibody-coated well. Discard the red-marked wells.
- (12) Mix by sliding the wells back and forth on a flat surface in a manner to ensure adequate mixing for 10-20 seconds, without splashing reagents, and incubate for 2 minutes.
- (13) Fill each well with distilled or deionized water and shake out. Repeat 5 times. Remove all water droplets by turning wells upside down and vigorously tapping on an absorbent paper.
- (14) Using a new tip, add 100 µL of substrate from the green-labeled bottle to each well. Discard the tip.
- (15) Mix by sliding wells back and forth on a flat surface for 10-20 seconds, and incubate for 3 minutes.

- (16) Using a new tip, add 100 µL of Red Stop from the red-labeled bottle to each well. Discard the tip.
- (17) Visually check that each well is one homogeneous color and no striations in color are present. Mix gently if needed to ensure thorough mixing within each well.

b. Interpretation of the Results

- (1) If a sample well is as blue as or darker than the control well, the sample contains less than 20 ppb of aflatoxins.
- (2) If a sample well shows less blue color, or more red color, than the control, the sample contains greater than or equal to 20 ppb of aflatoxins.
- (3) For optimum observation of color differences, place the wells on a white surface and read looking down through the solution.

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

The kit can be used until the expiration date on the label when stored refrigerated at 2-8°C (35-46°F)

b. Precautions

- (1) Methanol solution is 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) Store test kit between 2-8°C (35-46°F) when not in use, do not freeze test kits and avoid prolonged storage of kits at ambient temperatures.
- (3) **Follow GIPSA-issued instruction to run the test.**
- (4) Do not use kit components beyond expiration date.
- (5) Do not mix reagents from one lot of kit with another.

- (6) Do not run more than 4 tests per batch, unless using multichannel pipettor.
- (7) Follow proper pipetting techniques, including priming pipette tips by filling and dispensing solution once before use.
- (8) Use of incubation times other than those specified may give inaccurate results
- (9) Bring kits to room temperature (18-30°C, 64-86°F) prior to use.
- (10) Treat all used liquids, including sample extract, and labware as if contaminated with aflatoxins. Use precaution when handling
- (11) To avoid cross-contamination, use new pipette tips for each measurement, and thoroughly detoxify and wash all glassware between samples.
- (12) Do not use substrate that has turned blue prior to use.

EQUIPMENT AND SUPPLIES

a. Materials Provided in Test Kits (24 well kit).

- (1) 24 antibody-coated microwells
- (2) 24 red-marked mixing wells
- (3) 1 yellow-labeled bottle of 20 ppb Aflatoxin control
- (4) 1 blue-labeled bottle of Aflatoxin-HRP conjugate solution
- (5) 1 green-labeled bottle of K-Blue Substrate solution
- (6) 1 red-labeled bottle of Red Stop solution

b. Materials required but not provided.

- (1) Extraction materials (items i through iv available in kit form from Neogen item #8052)
 - i. 70% ACS methanol solution (Neogen item #8055/8056)
 - ii. Neogen filter syringe, (Neogen item #9420)
 - iii. Sample collection tubes (Neogen item #9421)
- (2) 1000, 500, 250 mL graduated cylinder
- (3) Scale capable of weighing 50 grams
- (4) 100 µL pipettor (Neogen item #9272/#9278)

- (5) Tips for 100 μ L pipettor (Neogen item #9410/#9407)
- (6) Paper towels or equivalent absorbent material
- (7) Plastic bucket for use as a waste receptacle
- (8) Microwell holder (Neogen item #9402)
- (9) Timer (Neogen item #9426)
- (10) Waterproof marker
- (11) Wash bottle (Neogen item #9400)
- (12) Distilled or deionized water

REVISION HISTORY

Revision 0 (02/18/2015)