

Read instructions carefully before starting test

Agri-Screen®

Aflatoxin Screening Test

USDA-GIPSA 2010-006

REFRIGERATE AT 2–8°C • DO NOT FREEZE

THE TOXIN

Aflatoxin is a toxic and carcinogenic substance produced by certain strains of the molds *Aspergillus flavus* and *A. parasiticus*. There are four principle types of aflatoxin: B₁, B₂, G₁ and G₂. Aflatoxin B₁ is the most frequently encountered of the group and the most toxic. The commodities most affected by aflatoxin are corn, peanuts, cottonseed, milo, and the majority of tree nuts.

The affects in animals of ingesting excessive amounts of the toxin range from chronic health and performance problems to death. Aflatoxin has been shown to cause liver damage or cancer, decreased milk and egg production, immune suppression and interference with reproductive efficiency.

The FDA has set maximum allowable levels of aflatoxin in food and feed. Therefore, accurate determination of the presence of the toxin is of major importance to those monitoring the quality of food and feed in which aflatoxin may occur. Testing these commodities for the toxin requires careful sampling, chemical extraction, sanitation and quantitative analysis.

The FDA has issued regulatory levels for aflatoxin as follows:

For	Level	Commodities
Humans	20 ppb	All food except milk
All animal species	20 ppb	All feed (exceptions below)
Exceptions: Breeding cattle, Breeding swine, Mature poultry	100 ppb	Corn
Finishing swine (>100 lbs)	200 ppb	Corn
Finishing beef cattle	200 ppb	Corn
Finishing beef cattle, swine poultry	300 ppb	Cottonseed meal

INTENDED USE

Agri-Screen for Aflatoxin is intended for the screening for aflatoxin in commodities like corn, cottonseed, milo, peanuts, popcorn, rice, wheat, and soy.

INTENDED USER

The test kit is designed for use by quality control personnel and others familiar with food and feed possibly contaminated by aflatoxin. Since technique is very important, operators should be trained by a Neogen representative or someone who has completed the Neogen training.

ASSAY PRINCIPLES

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 parts per billion (ppb). A methanol/water solution is used to extract any existing aflatoxin from a ground sample. Extracted aflatoxin in the 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 indicates strong positives.

STORAGE REQUIREMENTS

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

MATERIALS PROVIDED

- 24 antibody-coated microwells
- 24 red-marked mixing wells
- 1 yellow-labeled bottle of 20 ppb aflatoxin control (see precautions for handling of methanol solution)
- 1 blue-labeled bottle of aflatoxin-HRP conjugate solution
- 1 green-labeled bottle of K-Blue® Substrate solution
- 1 red-labeled bottle of Red Stop solution

MATERIALS RECOMMENDED BUT NOT PROVIDED

- Extraction materials (items b through d available in kit form from Neogen, item #8052):
 - 70% ACS methanol solution (Neogen item #8055/8056)
 - Container with 125 mL capacity (Neogen item #9428)
 - Neogen filter syringes, Whatman #1 filter paper, or equivalent (Neogen item #9420/9430)
 - Sample collection tubes (Neogen item #9421)
- Graduated cylinder, 50 mL (Neogen item #9447)
- Blender, high-speed (Neogen item #9493)
- Agri-Grind grinder or equivalent (Neogen item #9401)
- Scale capable of weighing 5-50 grams (Neogen item #9427)
- Pipettor, 100 µL (Neogen item #9272 or 9278)
- Pipettor tips for 100 µL pipettor (Neogen #9410)
- Paper towels or equivalent absorbent material
- Plastic bucket for use as waste receptacle
- Microwell holder (Neogen item #9402)
- Timer (Neogen item #9426)
- Waterproof marker
- Wash bottle (Neogen item #9400)
- Distilled or deionized water

PRECAUTIONS

- 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.

- 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.
- Do not use kit components beyond expiration date.
- Kits should be at room temperature (18–30°C, 64–86°F) prior to use.
- Do not mix reagents from one kit serial with reagents from a different kit serial.
- Do not run more than 4 wells per test, unless using a multichannel pipettor.
- Follow proper pipetting techniques, including priming pipette tips by filling and dispensing solution once before use.
- Use of incubation times other than those specified may give inaccurate results.
- 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.
- To avoid cross-contamination, use clean pipette tips and glassware for each sample, and thoroughly wash all glassware between samples.
- Commodities tested should have a pH of 6-8. Excessively acidic or alkaline samples should be adjusted. For instructions on adjusting pH contact your Neogen representative or Technical Services.
- Do not use substrate that has turned blue prior to use.

CHANGING THIS TEST KIT'S SCREENING LEVEL

This test kit can be used to screen samples for aflatoxin at levels other than the 20 ppb supplied control. Contact Neogen for more information on screening samples at a level other than 20 ppb.

SAMPLE PREPARATION AND EXTRACTION

The sample to be tested should be collected according to accepted sampling techniques. Store samples at 2–8°C (35–46°F) until analyzed.

- If not using Neogen's prepared solution, prepare a 70% methanol solution by mixing 7 parts ACS Grade methanol with 3 parts distilled or deionized water for each sample to be tested.
- Obtain a representative sample. Grind the sample so at least 75% of the ground material passes through a 20 mesh sieve, about the particle size of fine instant coffee.
- Combine at a ratio of 1:5. For example, mix 5 grams of ground sample with 25 mL 70% methanol and shake vigorously for 3 minutes or blend for 1 minute. **USDA/GIPSA Method:** Combine 50 grams of ground sample with 250 mL 70% methanol and blend for 1 minute or shake vigorously for 3 minutes.
- Filter at least 5 mL of the extract by using a Neogen filter syringe or pouring through a Whatman #1 filter. Collect the filtrate as the sample.
- The sample is now ready for testing.

TEST PROCEDURE

Allow all reagents to warm to room temperature (18–30°C, 64–86°F) prior to use.

- 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.
- Remove an equal number of antibody-coated wells. Return wells which will not be used immediately 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.
- Mix each reagent by swirling the reagent bottle prior to use.
- Using a new pipette tip, add 100 µL of conjugate from the blue-labeled bottle to each red-marked mixing well. Discard the tip.
- Using a new tip, add 100 µL of the control from the yellow-labeled bottle to the first well of the red-marked strip. Thoroughly mix by inserting the tip in the liquid and pipetting up and down 5 times. Discard the tip.
- Using a new tip, add 100 µL of the first sample to the second red-marked well. Thoroughly mix by inserting the tip in the liquid and pipetting up and down 5 times. Discard the tip. Repeat the process for each additional sample in a following red-marked well.
- 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.

- Mix by sliding the wells back and forth on a flat surface in a manner to ensure adequate mixing for 10 to 20 seconds, without splashing reagents. Wait 2 minutes after mixing.
- The initial reaction is now completed. Shake out the contents of the wells.
- 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.
- Using a new tip, add 100 µL of substrate from the green-labeled bottle to each well. Discard the tip.
- Mix by sliding wells back and forth on a flat surface for 10 to 20 seconds, and wait 3 minutes after mixing.
- Using a new tip, add 100 µL of Red Stop from the red-labeled bottle to each well. Discard the tip. Mix as before. Visually check to confirm thorough mixing.

INTERPRETATION OF RESULTS

If a sample well is **as blue** or **darker blue** than the control well, the sample contains **less than 20 ppb** of aflatoxin. If a sample well shows **less blue** color, or **more red** color, than the control, the sample contains **more than 20 ppb** of aflatoxin. For optimum observation of color differences, place the wells on a white surface and read looking down through the solution.

Alternative: Microwells may be read in a microwell reader. Wipe the bottom of wells, blank reader on air using a 650 nm filter, and compare sample readings to the 20 ppb control reading.

RETESTING

If positives occur in commodities not previously tested, confirm with an additional approved method prior to taking action.

VALIDATED COMMODITIES

Ammoniated corn, ammoniated cottonseed meal*, barley, beet pulp*, coconut, copra, corn, corn bran (3 min blend), corn germ meal, corn gluten meal*, corn grits, corn meal, corn/soy blend, corn starch, cottonseed, cottonseed meal, DDGS (Afla HS special procedure), DDGs wet cake*, DDGs syrup*, Figs, flaxseed meal, hominy, kamut, lentils, milo (grain sorghum), oats, oat fiber, oat hulls*, oats (naked), peanut meal#, peanuts (raw)#, peanuts (roasted)#, peanut hulls, pet food*, popcorn, potato (white), pumpkin seeds, quinoa, rice, rice bran, rice gluten, rice hulls, rye, soy flour, soy germ meal, soy hydrolysate, soybean meal, sunflower meal, tapioca, wheat, wheat bran* and wheat midds.

* = A pH adjustment step may be necessary

= See Peanut Butter Extraction Procedure

For commodities not listed above, please contact your Neogen representative.

CUSTOMER SERVICE

Neogen Customer Assistance and Technical Service can be reached between 8 a.m. and 6 p.m. Eastern Time by calling 800/234-5333 or 517/372-9200 and asking for a Neogen sales representative or Technical Services. Assistance is available on a 24-hour basis by calling 800/234-5333. Training on equipment use for all Neogen test kits is available.

MSDS INFORMATION AVAILABLE

Material safety data sheets (MSDS) are available for this test kit, and all of Neogen's test kits, on Neogen's Web site at www.neogen.com, or by calling Neogen at 800/234-5333 or 517/372-9200.

WARRANTY

Neogen Corporation makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. If any materials are defective, Neogen will provide a replacement of the product. Buyer assumes all risk and liability resulting from the use of this product. There is no warranty of merchantability of this product, or of the fitness of the product for any purpose. Neogen shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product.

TESTING KITS AVAILABLE FROM NEOGEN

Natural Toxins

- Aflatoxin, DON, Ochratoxin, Zearalenone, T-2/HT-2 Toxins, Fumonisin, Histamine

Foodborne Bacteria

- E. coli* O157:H7, *Salmonella*, *Listeria*, *Listeria monocytogenes*, *Campylobacter*, *Staphylococcus aureus*

Sanitation

- ATP, Yeast and Mold, Total Plate Count, Generic *E. coli* and Total Coliforms, Protein Residues

Food Allergens

- Peanuts, Milk, Eggs, Almonds, Gliadin, Soy, Hazelnut, Lupine, Mustard, Sesame, Shellfish, Walnut

Genetic Modification

- CP4 (Roundup Ready®)

Ruminant By-products

- Meat and Bone Meal, Feed



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