

Test Kit Instruction

April 11, 2018

Neogen Veratox Aflatoxin

FORWARD

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@ams.usda.gov.

Refer to the Mycotoxin Handbook for information on use of this test kit in official inspections including sampling, general sample preparation, 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@ams.usda.gov.

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1. GENERAL INFORMATION

VERATOX AFLATOXIN is a competitive direct enzyme-linked immunosorbent assay (CD-ELISA) which allows the user to obtain exact concentration of aflatoxins in parts per billion (ppb). Free aflatoxins in the samples and controls is allowed to compete with enzyme-labeled aflatoxin (conjugate) for the antibody binding sites. After a wash step, substrate is added, which reacts with the bound conjugate and produce blue color. More blue color means less aflatoxin in the sample. The test is read in a microwell reader to yield optical densities. The optical densities of the controls form the standard curve, and the sample optical densities are plotted against the standard curve to calculate the exact concentration of aflatoxins in the sample.

Approved Test Kit Information	
Test Kit Vendor:	Neogen Corporation 800/234-5333
Test Kit Name:	Veratox Aflatoxin
Product Number:	8030
Effective Date of Instructions:	4/11/2018
Instructions Revision Number:	0
Conformance Range:	5.0 – 300 ppb
Number of Analyses to Cover Conformance Range:	2
Type of Service:	Quantitative
Supplemental Analysis:	Yes
Approved Commodities:	corn (including dent or field corn, corn meal, corn flour, cracked corn, corn grits or polenta, and corn screenings), corn germ meal, corn gluten meal, corn/soy blend, distillers dried grains with solubles (DDGS), malted barley (including malted barley flour), brown rice, milled rice (including brewer's rice and glutinous rice), millet, pearl or pearled barley (including Quick Pearl Barley), popcorn, rice bran, rye (or rye berries, including whole grain rye flour, rye meal, cracked rye, and rye chops), sorghum, soybeans (including whole soybean and full-fat soy flour), white rye flour, wheat (including whole grain wheat flour, wheat middlings, wheat red dog, wheat flour 2nd clear, and wheat screenings), and wheat bran (including wheat bran aleurone)
Extraction method:	Blend 50 gram sample with 250 mL of 70% Methanol/30% distilled or deionized water (v/v) for 1 minute.
Test Format:	Competitive direct enzyme-linked immunosorbent assay
Detection Method:	Stat Fax Reader Model 321 Plus, Stat Fax Reader Model 4700

2. PREPARATION OF TESTING MATERIALS

a. Stat Fax Reader 321 Plus Set up.

- (1) Turn reader on and wait for the screen to say Ready.
- (2) Press Menu.
- (3) Select the Veratox Aflatoxin test by pressing the corresponding number on the keypad and press Enter.
- (4) Press 9 and enter then number of wells that will be tested and press Enter.
- (5) Press Enter to start reading the wells when ready.

b. Stat Fax Reader 4700 Set up.

- (1) Turn reader on and wait for the main menu to appear.
- (2) Press Run Test.
- (3) Select the Aflatoxin test from the menu.
- (4) Select Yes to accept that test.
- (5) Press # Wells and enter how many wells will be read then press OK.
- (6) Press Accept then Start when the wells are ready to be read.

c. Preparation of 1N Sodium Hydroxide (NaOH) Solution.

Note: One can buy premade 1N NaOH from any commercial supplier (e.g., Sigma Aldrich catalog# 72082) or may prepare from solid sodium hydroxide pellets (Sigma Aldrich Catalog# S8045) as described below.

- (1) Add slowly 4 grams of NaOH into 100 mL distilled or deionized water with stirring.
- (2) This solution should be used to adjust the pH of any sample extract that shows pH below 7.0.
- (3) Label the container stating the name, date of preparation and initials of technician that prepared the solution.
- (4) Store this solution at room temperature in a tightly closed container under fume hood.

CAUTION! NaOH is corrosive. Addition of solid NaOH pellets into water is an exothermic reaction (produces heat). Stir constantly and add the NaOH slowly.

d. Preparation of 1N Hydrochloric (HCl) Acid Solution.

Note: One can buy premade 1N HCl from any commercial supplier (e.g., Sigma Aldrich catalog #38283) or prepared from concentrated HCl (Sigma Aldrich catalog #320331) as described below.

- (1) Add slowly 8.2 mL of 12.1N HCl (concentrated Hydrochloric acid) into 91.8 mL distilled or deionized water with stirring.
- (2) This solution should be used to adjust the pH of any sample extract that shows pH above 8.0.
- (3) Label the container stating the name, date of preparation and initials of technician that prepared the solution.
- (4) Store this solution at room temperature in a tightly closed container under fume hood.

CAUTION! HCL is corrosive. Addition of concentrated acid into water is an exothermic reaction (produces heat). Stir constantly and add the HCl slowly.

3. SAMPLE PREPARATION AND EXTRACTION PROCEDURES

a. Preparation of sample.

The sample to be tested should be collected and prepared according to accepted sample techniques (see Mycotoxin Handbook).

b. Preparation of Extraction Solvent.

NOTE: If not using Neogen's premade extraction solvent, one can prepare a 70% methanol/30% deionized or distilled water solution (v/v) by mixing 7 parts ACS Grade methanol with 3 parts distilled or deionized water as described below.

- (1) 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.
- (2) Using a 500 mL graduated cylinder, add 300 mL of deionized or distilled water to the methanol and shake until completely mixed.
- (3) Label the container stating the mixture (70 percent methanol: 30 percent water), date of preparation and initials of technician that prepared the solution.
- (4) Store this solution at room temperature in a tightly closed container.
- (5) To prepare smaller or larger amounts of the solution use the ratio of 7 parts methanol to 3 parts distilled or deionized water.

c. Standard Extraction Procedure.

- (1) Weigh 50 ± 0.2 grams ground sample into a blender jar.
- (2) Using a 250 mL graduated cylinder, add 250 mL extraction solvent and put the lid on the jar.
- (3) Blend for 1 minute in a high-speed blender. Allow the mixture to settle for 1 minute.
- (4) Filter about 3 mL of the extract through a Neogen syringe filter.
- (5) For Corn Gluten Meal, DDGS and Wheat Bran, check the pH of the filtered extract using a pH paper. For all other commodities proceed to step 6.
 - (a) If the pH is not between 7.0 and 8.0, and if it is below 7.0, it needs to be adjusted.
 - (b) Using a disposable polyethylene transfer pipette, add one drop of 1N NaOH (sodium hydroxide) to the sample extract, vortex to mix, and check the pH.

- (c) If pH is still below 7.0, add another drop of 1N NaOH, mix, and check pH again. Continue this process until the pH falls between 7.0 and 8.0, then proceed to step 6.
- (d) The filtered extract is ready for testing and it can be used for testing for next 4 hours if it is kept in a tightly closed container.

4. TEST PROCEDURES

NOTE: For all unknown samples, analysis procedure A should be performed first. If the results are above 50 ppb, analysis procedure B should be followed. Results within the control range should be used for the result.

a. **Analysis Procedure (5.0 – 50 ppb Quantitation Range).**

- (1) Allow reagents and antibody wells to reach the room temperature (68 - 77°F) prior to running the test.
- (2) Remove 1 red-marked mixing well for each sample to be tested plus 4 red-marked wells for controls and place in the well holder.
- (3) Remove an equal number of antibody-coated wells. Return antibody wells, which will not be used immediately to the foil pack with desiccant and reseal to protect the antibody. Mark one end of strip with a “1” and place strip in the well holder with the marked end on the left. Do not mark the inside or bottom of the wells.
- (4) Mix each reagent by swirling the reagent bottle prior to use.
- (5) Using a 100 µL pipette, place 100 µL of conjugate from the blue-labeled bottle in each red-marked mixing well.
- (6) Using a new pipette tip for each, transfer 100 µL of controls and samples to the red-marked mixing wells.
- (7) Using a 12-channel pipette, mix the liquid in the wells by pipetting it up and down 3 times. Transfer 100 µL into the antibody-coated wells.
- (8) Mix by sliding the microwell holder back and forth on a flat surface for 10-20 seconds without splashing the reagents from the wells. Incubate for 2 minutes at room temperature (68 – 86°F). Discard red-marked mixing wells.
- (9) Shake out the contents of the antibody wells. Fill the wells with distilled or deionized water and dump them out. Repeat this step 5 times, then turn the wells upside-down and tap out on a paper towel until the remaining water has been removed.

- (10) Pour the needed volume of substrate from the brown-labeled bottle into the reagent boat.
- (11) With new tips on the 12-channel pipette, prime and pipette 100 µL of substrate into the wells and mix by sliding back and forth on a flat surface for 10-20 seconds. Discard the tips. Incubate for 3 minutes.
- (12) Discard remaining substrate and rinse the reagent boat with water.
- (13) Pour Red Stop solution from the red-labeled bottle (same volume as the substrate) into the red-labeled reagent boat.
- (14) With new tips on the 12-channel pipette, prime and pipette 100 µL of Red Stop to each well. Mix by sliding back and forth on a flat surface to make sure there is no layering. Discard the tips.
- (15) Wipe the bottom of the microwells with a lint free Kim wipe and read on the Stat Fax reader using a 650-nm filter. Air bubbles should be eliminated, as they could affect analytical results. Results should be read within 20 minutes after the addition of Red Stop.
- (16) In order to accept the result, the R-Value should be ≥ 0.98 .

b. **Dilution Protocol A (50 – 300 ppb Quantitation Range).**

- (1) Using the **filtered extract** made in the **Standard Extraction Procedure** above, dilute the **filtered extract** 8-fold with 70% methanol to prepare **Diluted Extract A**.
- (2) Using a 100 µL pipette, add 100 µL of **filtered extract** to 700 µL (measured using a 100 – 1000 µL variable volume pipette) of 70% methanol. This is **Diluted Extract A**. Vortex for a few seconds prior to analysis.
- (3) Follow the same test procedure as described in “**a. Analysis procedure (5.0 – 50 ppb Quantitation Range)**” using **Diluted Extract A** as the sample.
- (4) The result of this **Diluted Extract A** must be multiplied by 8 to obtain the final Aflatoxin concentration of the original test sample. Results following this protocol are valid in the range of 50 – 300 ppb.

5. 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@ams.uds.gov).

6. 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) Store test kit between 2-8°C (35-46°F) when not in use, do not freeze.
- (2) Do not use kit components beyond expiration date.
- (3) Do not mix reagents from one kit serial with reagents from a different kit serial. However, substrate and stopping solution are not lot specific.
- (4) Do not run more than 24 wells per test.
- (5) Follow proper pipetting techniques, including priming pipette tips by filling and dispensing solution once before use.
- (6) Use of incubation times other than those specified may give inaccurate results.
- (7) Bring kits to room temperature (68-77°F) prior to use.
- (8) Avoid prolonged storage of kits at ambient temperatures.
- (9) Treat all used liquids, including sample extract, and lab ware as if contaminated with aflatoxin. Gloves and other protective apparel should be worn at all times.
- (10) To avoid cross-contamination, use new pipette tips for each sample, and thoroughly detoxify and wash all glassware between samples.
- (11) 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.
- (12) 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.

7. EQUIPMENT AND SUPPLIES

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

- (1) 48 antibody-coated microwells
- (2) 48 red-marked mixing wells
- (3) 4 yellow-labeled bottles of 0, 5, 15, and 50 ppb Aflatoxin controls
- (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
- (7) Directions for use

b. Materials required but not provided.

- (1) Extraction solvent is available from Neogen, item #8052)
 - (a) 70% ACS Grade methanol (Neogen item #8055)
 - (b) 250 mL graduated cylinder (Neogen item #9368)
 - (c) Container with 125 mL capacity (250 mL for FGIS method)
 - (d) Neogen filter syringe, (Neogen item #9420)
 - (e) Sample collection tubes (Neogen item #9421)
- (2) 500 mL and 1000 mL graduated cylinders, Class A, calibrated to dispense. For preparing extraction solvent if not purchased premixed from Neogen.
- (3) High-speed blender
- (4) Agri-Grind grinder or equivalent (Neogen item #9401)
- (5) Scale capable of weighing 50 grams (Neogen item #9427)
- (6) 100 μ L pipettor (Neogen item #9272/#9278)
- (7) 12-channel pipettor (Neogen item #9273)
- (8) Tips for 12-channel and 100 μ L pipettor (Neogen item #9410/#9407)
- (9) Paper towels or equivalent absorbent material

- (10) Plastic bucket for use as a waste receptacle
- (11) Microwell holder (Neogen item #9402)
- (12) Timer (Neogen item #9426)
- (13) Waterproof marker
- (14) Wash bottle (Neogen item #9400)
- (15) 2 reagent boats for 12-channel pipettor (Neogen item #9435)
- (16) Distilled or deionized water
- (17) Microwell reader with a 650 nm filter (Neogen item #9302/#9303)
- (18) pH paper (Neogen item #9478)
- (19) Sodium Hydroxide pellets (NaOH) Sigma Aldrich Catalog# S8045

8. REVISION HISTORY

Revision 0 (4/11/2018)