

Test Kit Instruction

August 29, 2016

Neogen Corporation VERATOX for Total 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@usda.gov.

Refer to the Mycotoxin Handbook 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.

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1. GENERAL INFORMATION

VERATOX for TOTAL AFLATOXIN is a competitive direct enzyme-linked immunosorbent assay (CD-ELISA) which allows the user to obtain exact concentrations in parts per billion (ppb). Free aflatoxin 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 to produce a blue color. More blue color means less aflatoxin. 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 curve to calculate the exact concentration of aflatoxin.

Approved Test Kit Information	
Test Kit Vendor:	<i>Neogen Corporation 800/234-5333</i>
Test Kit Name:	VERATOX for Total Aflatoxin
Product Number:	8035
Effective Date of Instructions:	8/29/2016
Instructions Revision Number:	0
Conformance Range:	5.0 – 300 ppb
Number of Analyses to Cover Conformance Range:	2
Type of Service:	Quantitative
Approved Commodities:	Corn (including dent or field corn, corn meal, corn flour, cracked corn, corn grits or polenta, and corn screenings), pearled barley, corn gluten meal, distiller dried grains with solubles, popcorn, rye, sorghum, soybeans, and wheat.
Extraction method:	Vigorously shake 50g sample and one packet of MAX-2 G50 extraction powder in 250 mL of distilled or deionized water for 3 minutes.
Test Format:	Competitive direct enzyme-linked immunosorbent assay
Detection Method:	Stat Fax Reader Model 321 Plus, Stat Fax Reader Model 4700

2. PREPARATION OF READERS

a. Stat Fax 321 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 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.

3. SAMPLE PREPARATION AND EXTRACTION PROCEDURES

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

a. Sample Extraction Procedures (5-50 ppb quantitation range).

- (1) Weigh 50 ± 0.2 grams ground sample into a whirl pak bag.
- (2) Add one packet of MAX 2-G50 Aqueous Extraction Powder to the bag.

(CAUTION: Do not inhale the powder. Perform this step in a vent hood if possible.)

- (3) Add 250 mL of distilled or deionized water.
- (4) Shake vigorously by mechanical shaker (250 rpm) for 3 minutes.

- (5) Allow the sample to settle for 5 minutes.
- (6) Filter about 5 mL of the extract through a Neogen syringe filter. The filtered extract should be free of particulates.

The filtered extract is now ready for testing or dilution, as applicable.

b. Diluted sample extract (for quantitation range 50-300 ppb).

For samples with levels known to be between 50 and 300 ppb (or have tested above 50 ppb), a dilution needs to be made. Determine the appropriate dilution factor for the sample based on testing of the filtered extract or on expected concentration.

For a quantitation range of **50-300 ppb**, a ten-fold dilution is required. Dilute the samples as follows:

- (1) Pipette 100 µL of filtered extract into a suitable tube.
- (2) Add 900 µL of the sample diluent, and vortex to mix well.

The **diluted sample extract** is now ready for testing, and in this example Dilution Factor is 10.

Dilution Factor, DF = $((900\ \mu\text{L} + 100\ \mu\text{L}) / (100\ \mu\text{L}))$

4. TEST PROCEDURES

- a. Allow reagents and antibody wells to reach room temperature (68 - 77°F) prior to running the test. Also, mix each reagent by swirling the reagent bottle prior to use.
- b. Remove the appropriate number of red-marked mixing wells from the foil packet, and place in a well holder. Use four wells for the control samples plus one for each sample to be tested.
- c. Remove an equal number of antibody-coated wells from the foil packet, and add them to the well holder. 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.

(NOTE: Return antibody wells which will not be used immediately to the foil pack with desiccant and reseal to protect the antibody.)

- d. Place 100 µL of conjugate from the blue-labeled bottle into each red-marked mixing well.

- e. Using a new pipette tip for each, transfer 100 µL of each control and test sample (filtered extract or diluted sample extract) to the red-marked mixing wells. Controls should be in the first four wells, in ascending order, and the test samples should follow in the remaining wells.
- f. Using a 12-channel pipettor, mix the liquid in the wells by pipetting it up and down 3 times. Transfer 100 µL of this mixture to the antibody-coated wells.
- g. Incubate for 5 minutes at room temperature (10 – 30°C, 64 – 86°F). Mix by sliding the microwell holder back and forth on a flat surface for the first 30 seconds without splashing the reagents from the wells. Discard red-marked mixing wells.
- h. After the 5-minute incubation, shake out the contents of the antibody wells. Fill the wells with distilled or deionized water and then shake the water 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.
- i. Pour the needed volume of substrate from the green-labeled bottle into the green-labeled reagent boat.
- j. With new tips on the 12-channel pipettor, prime and pipette 100 µL of substrate into the wells.
- k. Incubate for 5 minutes. Mix by sliding back and forth on a flat surface for the first 30 seconds. Discard remaining substrate and rinse the reagent boat with water.
- l. Pour Red Stop solution from the red-labeled bottle (same volume as the substrate) into the red-labeled reagent boat.
- m. With new tips on the 12-channel pipettor, prime and pipette 100 µL of Red Stop Solution into each well. Mix well by sliding back and forth on a flat surface.
- n. Wipe the bottom of the microwells with a dry cloth or towel 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. An r-value of ≥ 0.980 is required for acceptable results.
- o. If the diluted filtered extract was used in the test, the displayed result must be multiplied by the dilution factor to obtain the final result for the sample.

5. REPORTING AND CERTIFYING TEST RESULTS

Refer to the Mycotoxin Handbook for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-659-8403 or Patrick.J.McCluskey@udsa.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 lot with reagents from a different kit lot.
- (4) Do not analyze or read more than 24 wells at a time.
- (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 (18-30°C, 64-86°F) prior to use.
- (8) Avoid prolonged storage of kits at ambient temperatures.
- (9) 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.
- (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.

7. EQUIPMENT AND SUPPLIES

a. Materials provided in test kits.

- (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) 1 pink-labeled bottle of dilution diluent

b. Materials required but not provided.

- (1) Extraction materials
 - (a) MAX-2 G50 Aqueous Extraction Packets (Neogen item #8036G)
 - (b) 250 mL graduated cylinder (Neogen item #9368, 9448)
 - (c) Container with 500 mL capacity
 - (d) Neogen filter syringe, Whatman #1 filter paper, or equivalent (Neogen item #9420, 9519, 9429)
 - (e) Sample collection tubes (Neogen item #9421)
- (2) Agri-Grind grinder or equivalent (Neogen item #9401)
- (3) Scale capable of weighing 50 grams (Neogen item #9427)
- (4) 100 μ L pipettor (Neogen item #9272/#9278)
- (5) 12-channel pipettor (Neogen item #9273)
- (6) Tips for 12-channel and 100 μ L pipettors (Neogen item #9410/#9407)
- (7) Paper towels or equivalent absorbent material
- (8) Plastic bucket for use as a waste receptacle
- (9) Microwell holder (Neogen item #9402)
- (10) Timer (Neogen item #9426)
- (11) Waterproof marker
- (12) Wash bottle (Neogen item #9400)
- (13) 2 reagent boats for 12-channel pipettor (Neogen item #9435)
- (14) Distilled or deionized water
- (15) Microwell reader with a 650 nm filter (Neogen item #9302/#9303)

8. REVISION HISTORY

Revision 0 (08/29/2016)