

HYGIENA

MycoTox Total Aflatoxin ELISA

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, grinding, reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey of PPMB by phone at 816-659-8403 or email at Patrick.J.McCluskey@usda.gov.

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1. GENERAL INFORMATION

Mycotox Total Aflatoxin is a competitive direct enzyme-linked immunosorbent assay intended for the quantitative detection of aflatoxins in corn. An aflatoxin specific antibody is coated to a polystyrene microwell. Aflatoxins are extracted from a ground sample with 70% methanol. The extracted sample and HRP-conjugated aflatoxin are mixed and added to the antibody-coated microwell. Aflatoxin from the extracted sample and HRP-conjugated aflatoxin compete to bind with the antibody coated to the microwell. Microwell contents are decanted and non-specific reactants are removed by washing. An enzyme substrate (TMB) is added and color (blue) develops. The intensity of the color is directly proportional to the amount of bound conjugate and inversely proportional to the concentration of aflatoxin in the sample or standard. Therefore, as the concentration of aflatoxin in the sample or standard increases, the intensity of the blue color will decrease. An acidic stop solution is added which changes the chromogen color from blue to yellow. The microwells are measured optically by a microplate reader with an absorbance filter of 450nm (OD₄₅₀). The optical densities of the samples are compared to the OD's of the kit standards and an interpretative result is determined.

Approved Test Kit Information	
Test Kit Vendor	Hygiena LLC. Phone# 714 578-7830
Test Kit Name:	Mycotox Total Aflatoxin ELISA
Product Number:	941AFL01G-96
Effective Date of Instructions:	9/30/2019
Conformance Range:	5.0 – 300 ppb
Number of Analyses to Cover Conformance Range:	1
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)
Extraction Method:	Blend 50 grams sample for 3 minutes with 250 mL of 70% methanol/30% distilled or deionized water (v/v) warmed at 40°C
Test Format:	Competitive enzyme immunoassay
Detection Method:	BioTek 800TS Microplate Reader, Model 800TS

2. PREPARATION OF TESTING MATERIALS

a. BioTek 800TS Reader Set up

- (1). Turn on the reader with the switch on the right side.
- (2). Turn on the computer and open GEN 5 software.
- (3). Choose Experiments and Create using an existing protocol.
- (4). Open Mycotox Total Aflatoxin file.
- (5). Click Read New icon (white arrow with green circle) to start reading the wells when ready.

b. Wash Solution

Dissolve the content of PBS-Tween packet in 1 liter of distilled or deionized water.

3. SAMPLE PREPARATION AND EXTRACTION PROCEDURES

Bring all reagents and samples to room temperature (20 – 25 °C / 68 – 77 °F) before use, and perform the sample preparation at room temperature.

a. Preparation of Sample

Collect and prepare all test samples according to accepted sampling techniques (See Mycotoxin Handbook).

b. Preparation of Extraction Solvent

- (1). Using a 1000 mL graduated cylinder, measure 700 mL of methanol (ACS grade or better) and pour it into a glass bottle.
- (2). Using a 500 mL graduated cylinder, add 300 mL of distilled or deionized water to the methanol and shake until completely mixed.
- (3). Label the glass bottle stating 70% methanol/30% water, date of preparation and initials of technician who prepared.
- (4). To prepare smaller or larger amounts of the extraction solvent, use the ratio of 7 parts methanol to 3 parts distilled or deionized water.
- (5). Place the extraction solvent bottle in a water bath with a temperature set to 40 °C and let it sit at least 1 hour before use. Use a thermometer to check the water bath temperature.

c. Extraction Procedure

- (1). Weigh 50 ± 0.2 grams ground sample into a 16 fluid oz. Mason jar.
- (2). Using 250 mL graduated cylinder, add 250 mL of warmed extraction solvent (70% methanol). Return the solvent extraction bottle to the warm water bath between samples.
- (3). Blend for 3 minutes at high speed. Extraction should be performed immediately so that extraction solvent temperature is close to 40 °C. Between samples, clean the Mason jar and blender parts with detergent, warm tap water and brush. Rinse with tap water.
- (4). Using a 1000 μ L pipette, transfer 1000 μ L into a microcentrifuge tube and centrifuge for 10 seconds.
- (5). Using a 1000 μ L pipette and new pipette tip, dispense 300 μ L of 70% methanol in a microtube.
- (6). Using a 100 μ L pipette, add 100 μ L of the supernatant in the microtube. Vortex for a few seconds to mix prior to analysis.

4. TEST PROCEDURES

Do not analyze more than two strips per run.

- a. Bring all reagents and samples to room temperature (20 – 25 °C / 68 – 77 °F) before use and perform the sample preparation at room temperature.
- b. Remove 1 red-marked mixing well for each sample and another 6 red-marked mixing wells for 6 standards.
- c. Remove an equal number of antibody-coated wells, and return unused wells to the foil pack with desiccant.
- d. Mix each reagent by swirling the reagent bottle prior to use.
- e. Using a 200 μ L pipette, dispense 200 μ L of conjugate (green capped bottle) into each red-marked mixing well.
- f. Using a 100 μ L pipette with a new pipette tip for each, add 100 μ L of standards and samples to the red-marked mixing wells.

- g. Using an 8-channel pipette, mix the liquid in the wells by pipetting it up and down 3 times. Transfer 100 µL into the antibody-coated wells.
- h. Incubate for 15 minutes at room temperature (20 – 25 °C / 68 – 77 °F).
- i. Discard the contents from the wells into a discard basin. Using a wash bottle, fill the wells with PBS-Tween wash buffer, then dump the buffer out of the wells into a discard basin. Repeat this step four more times.
- j. Tap the wells (face down) on a layer of absorbent towels to remove residual buffer.
- k. Using an 8-channel pipette, add 100 µL of substrate reagent (blue capped bottle) to each well. Incubate at room temperature for 5 minutes. Cover to avoid direct light.
- l. Using an 8-channel pipette, add 100 µL of stop solution (red capped bottle) in the same sequence and at the same pace as the substrate reagent was added. Mix gently by sliding the plate back and forth on a flat surface for 10 – 15 seconds.
- m. Wipe the bottom of the wells with a lint free Kimwipe and remove air bubbles.
- n. Read the optical density (OD) at 450 nm using the BioTek 800 TS reader. Read within 10 minutes after addition of stop solution.

5. READING THE RESULTS

- a. Using the BioTek 800 TS microplate reader:
 - (1). Load a plate with wells on the reader to read OD.
 - (2). Click Read New to begin reading.
 - (3). Once reading is complete, click Statistics and choose [Concentration] [Dilution] in Data option.
 - (4). Aflatoxin concentration in ppb is calculated and displayed in [Concentration][Dilution] column.
 - (5). An R^2 value ≥ 0.980 is required to accept the results. The value is shown in Graphs tab by choosing StdCurve Fitting Results.

6. 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@usda.gov).

7. STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions

The reagents can be stored until the expiration data on the kit label when stored at 2 – 8 °C.

b. Precautions

- (1). Bring all reagents to room temperature (20 – 25 °C / 68 – 77 °F) before use.
- (2). Do not use kit components beyond expiration date.
- (3). Return all reagents to 2 – 8 °C (35 – 46 °F) immediately after use.
- (4). Do not interchange reagents kits of different lot numbers.
- (5). Adhere to all time and temperature conditions stated in the procedure.
- (6). Never pipette reagents or samples by mouth.
- (7). Standards are flammable. Caution should be taken in the use and storage of these reagents.
- (8). Stop solution contains acid. Do not allow to contact skin or eyes. If exposed, flush with water.
- (9). Consider all materials, containers and devices that are exposed to sample or standards to be contaminated with aflatoxin. Wear protective gloves and safety glasses when using this kit.
- (10). Dispose of all materials, containers and devices in the appropriate receptacle after use.
- (11). HRP-labeled conjugate and TMB-substrate are photosensitive and are packaged in a protective opaque bottle. Store in the dark and return to storage after use.

8. EQUIPMENT AND SUPPLIES

a. Materials provided in test kits

- (1). 96 antibody coated wells

- (2). 96 red-marked mixing wells
- (3). 6 black-capped amber bottles of 0, 0.2, 0.6, 1.8, 5.0, and 15.0 ppb aflatoxin standards
- (4). 2 green-capped bottle of 12 mL conjugate
- (5). 1 blue-capped bottle of 12 mL substrate
- (6). 1 red-capped bottle of 12 mL stop solution
- (7). 1 packet of PBS-Tween wash buffer

b. Materials not provided in test kits

- (1). BioTek 800TS microplate reader (BioTek Catalog# 800TS)
- (2). 100 μ L and 1000 μ L pipettes (VWR Catalog# 89079-970 and 89079-974)
- (3). 8-channel pipette for 30 – 300 μ L (Thermo Scientific Catalog# 4661030N)
- (4). 100 μ L and 1000 μ L pipette tips (Phenix Research Catalog# TS-2776 and TS-125BRS)
- (5). Graduated cylinders (250 mL and 1000 mL)
- (6). 1.5 mL microcentrifuge tube (Phenix Research Catalog# MH-815)
- (7). Micro centrifuge (3000 rpm; Qualitron Catalog# DW-41-115)
- (8). Microtube (Phenix Research Catalog# B1268)
- (9). Balance and timer (Accuris Instruments Catalog# W3100A-120)
- (10). Water bath (Baxter Catalog# B6990)
- (11). Wash bottle (VWR Catalog# 16651-493)
- (12). Distilled or deionized water
- (13). ACS grade methanol (Fisher Scientific Catalog# LC1680044)
- (14). High-speed blender and Mason jar (16 fluid oz.) (Oster Catalog# BLSTSG-W00-000)
- (15). Reagent boats (Vista Lab Catalog# 3054-2002)
- (16). Paper towel
- (17). Kim wipe (Fisher Scientific Catalog# NC9855580)

(18). Thermometer capable of measuring 40°C for checking water bath

(19). Detergent (Alconox Tergazyme #1304)

9. REVISION HISTORY

Effective: 9/30/2019