Mycotox Total Aflatoxin

ELISA Quantitative

Catalog # 941AFL01G-96

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Quantitative ELISA

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For the quantitative detection of total aflatoxins in corn including dent or field corn, corn meal, corn flour, cracked corn, corn grits or polenta, and corn screenings.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

Contents

Introduction

Aflatoxins are toxic metabolites produced by a variety of molds such as *Aspergillus flavus* and *Aspergillus parasiticus*. They are carcinogenic and can be present in grains, nuts, cottonseeds and other commodities associated with human food or animal feeds. Crops may be contaminated by one or more of the four following sub-types of aflatoxin: B1, B2, G1 and G2. Aflatoxin B1 is the most toxic and frequently detected form. The other types present a significant danger if the concentration is at a high level. Aflatoxins have been implicated in human health disorders including hepatocellular carcinoma, aflatoxicosis, Reye's syndrome and chronic hepatitis. Animals are exposed to aflatoxins by consumption of feeds that are contaminated by aflatoxin producing fungal strains during growth, harvest or storage. Symptoms of toxicity in animals range from death to chronic diseases, reproductive interference, immune suppression, decreased milk and egg production. Most controlling government agencies worldwide have regulations regarding the amount of aflatoxins allowable in human and animal foodstuffs. Accurate and rapid determination of the presence of aflatoxin in commodities is of paramount importance.

The Hygiena Mycotox Total Aflatoxin ELISA kit was developed to determine aflatoxins with a wide range of 5 - 300 ppb in grains and certified by the Federal Grain Inspection Service (FGIS) for the quantitative determination of aflatoxins in corn (Certificate No. FGIS 2019-129).

Assay Principle

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.

Limitations Of The Procedure

- For research use only. Not for use in diagnostic procedures.
- Bring all reagents to room temperature (19° - 25°C) before use.
- Store reagents at 2°C to 8°C, and do not use beyond expiration date(s). Never freeze kit components.
- Do not return unused reagents back into their original bottles. The assay procedure details volumes required.
- Adhere to all time and temperature conditions stated in the procedure.

Precautions For Users

- Never pipette reagents or samples by mouth.
- The Stop Solution contains acid. Do not allow to contact skin or eyes. If exposed, flush with water.
- Standards are prepared in methanol, which is flammable. Keep reagents away from heat sources. Wear protective gloves when using this kit.
- Dispose of all materials, containers and devices in an appropriate receptacle after use.
- 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.

Reagents Provided

1X Pouch	**Antibody coated microwell plate**		96 wells (12 eight well holder) coated with a mouse anti-aflatoxin monoclonal antibody, *Ready-To-Use.*
1X Plate	**Mixing wells**	Red	96 non-coated wells (12 eight well strips) in a microwell holder, *Ready-To-Use.*
6X Vials	**Aflatoxin standards**	Black cap	1.5 mL/vial of aflatoxin at the following concentrations: 0.0, 0.2, 0.6, 1.8, 5.0, and 15.0 ng/mL in 70% Methanol, *Ready-To-Use.*
2X Bottle	**Aflatoxin-HRP Conjugate**	Green cap	2 x 12 mL of aflatoxin B1 conjugated peroxidase in buffer with perservative, *Ready-To-Use.*
1X Bottle	**Substrate reagent**	Blue cap	12 mL stabilized tetramethylbenzidine(TMB), *Ready-To-Use.*
1X Bottle	**Stop solution**	Red cap	12 mL Acidic Solution, *Ready-To-Use.*
1X Pouch	**Washing buffer**		PBS WITH 0.05% Tween20®, bring to 1 liter with distilled water and store refrigerated.

Materials Required But Not Provided

- Single or multi-channel pipettor with 100, 200, and 1000 µL tips
- Timer
- Wash bottle
- Absorbent paper towels
- Microcentrifuge and tubes
- Analytical balance
- Graduated cylinders (250 mL and 1000 mL)
- Microtube
- Water bath
- Vortex mixer
- Kitchen blender
- Mason jar (16 fluid oz.)
- Methanol (ACS grade)
- Distilled water
- Microplate reader equipped with a 450 nm filter (BioTek 800TS)

Preparation of Extraction Solvent

1. Using a 1000 mL graduated cylinder, measure 700 mL of methanol 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.

Note: The sample must be collected according to the appropriate established sampling techniques.

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.
4. Using a 1000 µL pipette, transfer 1000 µL into a microcentrifuge tube and centrifuge for 10 seconds.
5. Using a 1000 µL pipette and new pipette tip, dispense 300 µL of 70% methanol in a microtube.
6. Using a 100 µL pipette, add 100 µL of the supernatant in the microtube. Vortex for a few seconds to mix prior to analysis.

Assay Procedure

1. Bring all reagents and samples to room temperature (20 – 25 °C) before use and perform the sample preparation at room temperature.
2. Remove 1 red-marked mixing well for each sample and another 6 red-marked mixing wells for 6 standards.
3. Remove an equal number of antibody-coated wells, and return unused wells to the foil pack with desiccant.
4. Mix each reagent by swirling the reagent bottle prior to use.
5. Using a 200 µL pipette, dispense 200 µL of conjugate (green capped bottle) into each red-marked mixing well.
6. Using a 100 µL pipette with a new pipette tip for each, add 100 µL of standards and samples to the red-marked mixing wells.
7. 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.
8. Incubate for 15 minutes at room temperature.
9. 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.
10. Tap the wells (face down) on a layer of absorbent towels to remove residual buffer.
11. 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.
12. 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.
13. Wipe the bottom of the wells with a lint free Kimwipe and remove air bubbles.
14. Read the optical density (OD) at 450 nm. Read within 10 minutes after addition of stop solution.

Interpretation of Results

Construct a dose-response curve using either the unmodified OD values or the OD values expressed as a percentage (%B/Bo) of the OD of the zero (0.0) standard against the aflatoxin content of the standard. Unknowns are measured by interpolation from the standard curve.

The information contained on the label of a standard vial refers to the contents of that vial. However, the sample has been diluted at a 1:20 ratio with 70% methanol, and so the level of aflatoxin shown by the standard must be multiplied by 20 in order to indicate the ng of aflatoxin per gram of commodity (ppb) as follows:

Standard (ng/mL)	Commodity (ppb)
0.0	**0.0**
0.2	**4.0**
0.6	**12.0**
1.8	**36.0**
5.0	**100.0**
15.0	**300.0**

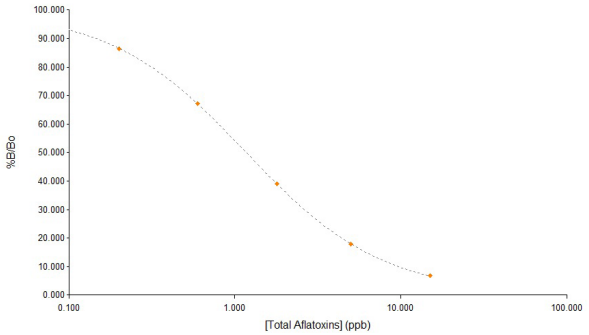
The sample dilution results in a standard curve from 4 ppb to 300 ppb. If a sample contains aflatoxin at greater concentration than the highest standard, it should be diluted appropriately in 70% methanol and retested. The extra dilution step should be taken into consideration when expressing the final result.

Assay Characteristics

Data from nine consecutive standard curves gave the following results.

Aflatoxin (ng/mL)	%B/B0	CV (%)
0.0	100	-
0.2	86	2.3
0.6	67	2.9
1.8	39	1.8
5.0	18	3.6
15.0	7	10.1

The graph below represents the data in the table above.



Accuracy on corn samples naturally contaminated with aflatoxins

(n = 21 per each concentration)

Aflatoxin in corn (ppb)	Average (ppb)	Standard deviation	Acceptable range (ppb)
5.2	5.27	0.60	2.6 - 7.8
18.3	18.13	1.26	11.0 - 25.6
87.9	80.88	6.51	59.8 - 116
300	243.46	7.48	204 - 396

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