Aflatoxin B1

ELISA Quantitative

Catalog # 941BAFL01B1-96

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For the quantitative detection of Aflatoxin B_1 in grains, nuts, cottonseeds, cereals and other commodities including animal feeds.

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, cottonseed and other commodities associated with human food or animal feed. Crops may be contaminated with aflatoxin B_1. Aflatoxin B_1 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.

Assay Principle

The Helica Aflatoxin B_1 Assay is a solid phase direct competitive enzyme immunoassay (ELISA). An aflatoxin specific antibody optimized to react with B_1 is coated to a polystyrene microwell. Toxins are extracted from a ground sample with 70% methanol. The extracted sample and HRP-conjugated Aflatoxin B_1 are mixed and added to the antibody-coated microwell. Aflatoxin from the extracted sample and HRP-conjugated Aflatoxin B_1 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 a blue color 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 chromagen 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 ODs 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.

Reagents Provided

1 x pouch	**Antibody coated microwell plate**	Silver pouch	96 wells (12 eight-well strips) in a microwell holder coated with a mouse anti-aflatoxin monoclonal antibody, *Ready-To-Use*
1 x plate	**Dilution wells**	Green	96 non-coated wells (12 eight-well strips) in a microwell holder, *Ready-To-Use*
6 x vials	**Aflatoxin standards**	Black cap	1.5mL/vial of aflatoxin B_1 at the following concentrations: 0.0, 0.2, 0.5, 1.0, 2.0, and 4.0ng/mL in organic solution, *Ready-To-Use*
2 x bottles	**Aflatoxin HRP-conjugate**	Green cap	2 x 12mL of aflatoxin B_1 conjugated to peroxidase in buffer with preservative, *Ready-To-Use*
1 x bottle	**Substrate reagent**	Blue cap	12mL stabilized tetramethylbenzidine (TMB), *Ready-To-Use*
1 x bottle	**Stop solution**	Red cap	12mL acidic solution, *Ready-To-Use*
1 x pouch	**Washing Buffer**	White pouch	PBS with 0.05% Tween20, bring to 1L with distilled water and store refrigerated

Materials Required But Not Provided

Extraction Procedure

- A grinder sufficient to render sample to particle size of fine instant coffee
- Collection container with minimum 125mL capacity
- Balance with 20g measuring capability
- Graduated cylinder - 100mL
- Methanol - 70mL reagent grade per sample
- Distilled or deionized water -30mL per sample
- Filter Paper - Whatman #1 or equivalent
- Filter Funnel

Assay Procedure

- Pipettor with tips of 100µL and 200µL
- Timer
- Wash bottle
- Absorbent paper towels
- Microplate reader with 450nm filter

Precautions For User

- Never pipette reagents or samples by mouth.
- Standards are flammable. Caution should be taken in the use and storage of these reagents.
- The stop solution contains acid. Do not allow to contact skin or eyes. If exposed, flush with water.
- Consider all materials, containers and devices that are exposed to sample or standards to be contaminated with aflatoxin. 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.

Extraction Procedure

The sample must be collected according to established sampling techniques

1. Prepare the Extraction Solution (70% Methanol) by adding 30mL of distilled or deionized water to 70mL of methanol (reagent grade) for each sample to be tested.
2. Grind a representative sample to the particle size of fine instant coffee (95% passes through a 20 mesh screen).
3. Weigh out a 20g ground portion of the sample and add 100mL of the Extraction Solvent (70% Methanol).

   The ratio of sample to extraction solvent is 1:5 (w/v).

4. Mix by shaking in a sealed container or in a blender for a minimum of 2 minutes.
5. Allow the particulate matter to settle, then filter 5 - 10mL of the extract through a Whatman #1 filter paper (or equivalent) and collect the filtrate to be tested. The sample is now ready for testing.

Assay Procedure

It is recommended that a multi-channel pipettor be utilized to perform the assay. If a single channel pipettor is used, it is recommended that no more than a total of 16 samples and standards (2 test strips) are run.

1. Bring all the reagents to room temperature before use. Reconstitute the PBS-Tween packet by washing out the contents with a gentle stream of distilled water into a 1-Liter container. Q.S. to 1 Liter with distilled water and store refrigerated when not in use.
2. Place one Dilution Well in a microwell holder for each Standard and Sample to be tested. Place an equal number of Antibody Coated Microtiter Wells in another microwell holder.
3. Dispense 200µL of the Conjugate into each Dilution Well.
4. Using a new pipette tip for each, add 100µL of each Standard and Sample to appropriate Dilution Well containing Conjugate. Mix by priming pipettor at least 3 times.

Operator must record the location of each Standard and Sample throughout test.

5. Using a new pipette tip for each, transfer 100µL of contents from each Dilution Well to a corresponding Antibody Coated Microtiter Well. Incubate at room temperature for 15 minutes.
6. Decant the contents from microwells into a discard basin. Wash the microwells by filling each with PBS-Tween wash buffer, then decanting the buffer into a discard basin. Repeat wash for a total of 5 washes.
7. Tap the microwells (face down) on a layer of absorbent towels to remove residual wash buffer.
8. Measure the required volume of Substrate Reagent (1mL/strip or 120µL/well) and place in a separate container. Add 100µL to each microwell. Incubate at room temperature for 5 minutes. Cover to avoid direct light.
9. Measure the required volume of Stop Solution (1mL/strip or 120µL well) and place in a separate container. Add 100µL in the same sequence and at the same pace as the Substrate was added.
10. Read the optical density (OD) of each microwell with a microtiter plate reader using a 450nm filter. Record the optical density (OD) of each microwell

Interpretation Of Results

Construct a dose-response curve using either the unmodified OD values or the OD values expressed as a percentage of the OD of the zero (0.0ng/mL) 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 5:1 ratio with 70% methanol, and so the level of aflatoxin shown by the standard must be multiplied by 5 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	1.0
0.5	2.5
1.0	5.0
2.0	10.0
4.0	20.0

The sample dilution results in a standard curve from 1ppb to 20ppb. If a sample contains aflatoxin at greater 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.

Performance Data

Within Assay Variation A typical example of the Helica Aflatoxin B_1 assay run in duplicate yielded the following standard curve and within assay variation.			
ppb in sample	**Mean OD**	**%B/B0**	**%CV**
0.0	1.835	100	1.9
1.0	1.626	88.6	1.5
2.5	1.283	69.9	1.0
5.0	0.720	39.2	2.4
10.0	0.338	18.4	1.0
20.0	0.147	8.0	1.0

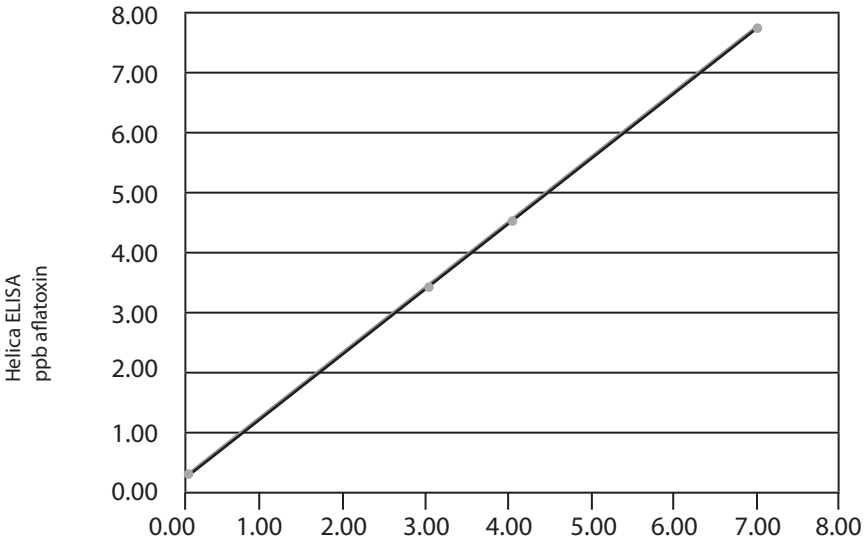
Between Assay Variation Between assay variation is expressed as percentage of B_0 for each standard. n= 6 assays.		
ppb in sample	**%B/Bo**	**%CV**
1.0	82.6	3.0
2.5	60.6	5.1
5.0	33.6	4.8
10.0	16.6	6.0
20.0	7.7	7.8

Limit of detection (LOD) is defined as the mean plus two standard deviations of multiple determinations of an Aflatoxin-free commodity extract. As different commodities generate somewhat different zeros due to 'matrix inhibition' effects, it follows that the LOD is commodity specific and should be measured empirically for each different commodity.

Using the Helica Aflatoxin B_1 assay:

LOD for corn in <1.0ppb n=10
LOD for peanuts is <1.0ppb n= 10

Aflatoxin Helica ELISA vs HPLC (maize - corn)



$y = 1.0448x + 0.2132$

$R^2 = 0.9998$

The Helica Aflatoxin B_1 ELISA has been tested at 1mg/mL (1 million ppb) without evidence of anomalous binding behavior (high-dose hook effect). *Therefore, it may be used to assess gross environmental contamination.*

Innovation Based On Integrated Science

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