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Aflatoxin M1

ELISA Quantitative (ULTRA)

Catalog # 961AFLM01C-ULTRA

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Catalog # 961AFLM01C-ULTRA

For the quantitative detection of Aflatoxin M_1 in milk, milk powder, and yogurt.

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 feeds. Crops may be contaminated by one or more of the four following sub-types of Aflatoxin: B_1, B_2, G_1 and G_2. 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. When cows are fed contaminated feed, Aflatoxin B_1 is converted by hydroxylation to Aflatoxin M_1, which is subsequently secreted in the milk of lactating cows. Aflatoxin M_1 is quite stable towards the normal milk processing methods such as pasteurization and if present in raw milk, it may persist into final products for human consumption.

Most controlling government agencies worldwide have regulations regarding the amount of aflatoxins allowable in human and animal foodstuffs. Many countries have declared limits for the presence of Aflatoxin M_1 in milk and milk products. In the EU the limit for the presence of M_1 in milk and reconstituted milk powders has been set at 0.05 μg/L or 50 parts per trillion (50 ppt).

Assay Principle

The HELICA Aflatoxin M_1 Assay is a solid phase competitive enzyme immunoassay. An antibody with a high affinity for Aflatoxin M_1 is coated onto polystyrene microwells. Standard or sample is added to the appropriate well and if Alatoxin M_1 is present it will bind to the coated antibody. Subsequently, aflatoxin bound to horse radish peroxidase (HRP) is added and binds to the antibody not already occupied by Aflatoxin M_1 present in the sample or standard. After this incubation period, the contents of the wells are decanted, washed and an HRP substrate is added which develops a blue color in the presence of an enzyme. The intensity of the color is directly proportional to the amount of bound conjugate and inversely proportional to the amount of Aflatoxin M_1 in the standard or sample. Therefore, as the concentration of Aflatoxin M_1 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 ODs of the kit standards and an interpolated 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

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 M1 standards**		4.0 mL/vial of Aflatoxin M1 at the following concentrations: 0.0, 10.0, 30.0, 80.0, 200.0, 500.0 pg/mL (ppt) in stabilized skim milk, *Ready-To-Use*.
1X Bottle	**Aflatoxin HRP-conjugate**	Green cap	12 mL of aflatoxin conjugated to horseradish peroxidase in buffer with preservative, *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.
1X Bottle	**M1 free skim milk**	White cap	12 mL of skim milk, *Ready-to-Us*e.

Materials Required But Not Provided

- Single or multi-channel pipettor with 100 and 200 µL tips
- Glass tubes
- Timer
- Wash bottle
- Absorbent paper towels
- Centrifuge
- Microplate reader with 450 nm filter

Precautions For User

- 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.
- Consider all materials, containers and devices that are exposed to sample or standards to be contaminated with Aflatoxin M_1. 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.

Preparation of Samples

Raw Milk

1. The standards are presented in homogenized skim milk and skim milk (milk plasma) is the appropriate sample for the assay.
2. An aliquot of unprocessed raw fatty milk should be placed at refrigerated temperature overnight to allow the fat globules to rise to the surface in a natural "creaming" effect. Centrifugation at this point is not necessary.
3. Alternatively, if the sample is at ambient temperature or has been mixed in transit, place an aliquot at refrigerated temperature for 1 – 2 hours and centrifuge at 2,000 g for 5 minutes to induce separation of the upper fatty layer.
4. Remove the upper fatty layer by aspiration and use the lower plasma in the assay.

Homogenized Milk

1. Homogenized skim milk should be used directly in the assay.
   Note: Due to the stabilization of the fat globules induced by the homogenizing process they are difficult to eliminate even by high speed centrifugation to create a plasma from homogenized fatty milk.
   (see recovery data on page 7).

Milk Powder

1. Reconstitute milk powders according to the manufacturer's instructions and treat the reconstituted product as above.

Yogurt

1. Weigh out an accurate amount of yogurt in a clean tube.
3. Add an equivalent amount of skim milk provided into the tube.
   (e.g. Add 1mL skim milk for 1g yogurt).
4. Vortex the tube about 30 seconds until the mixture is homogenized.
   It is ready to use in the assay.

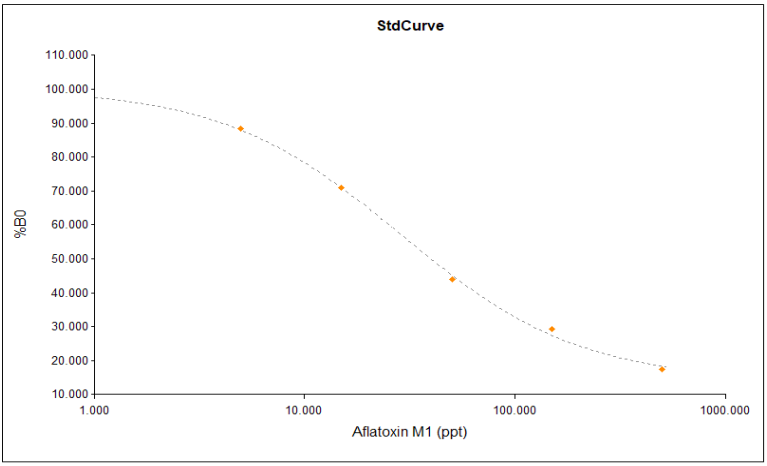
Assay Procedure

1. Bring 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.
2. Place one mixing 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. Aliquot 450 µL of standards and sample into microtubes.
4. Using a multichannel pipettor, transfer 200 µL aliquots of standards and samples from the microtubes into the appropriate Antibody Coated Wells in duplicate. If running singlets, scale the volume down accordingly.
5. Incubate at ambient temperature (19° - 25°C) for 20 minutes.
6. During the incubation, dispense 150 µL of standard or sample into each mixing well, and add 150 µL of the conjugate to each well. Mix by priming pipettor at least 3 times. If running singlets, scale the volume down accordingly. Note: Operator must record the location of each Standard and Sample throughout test.
7. After 20 minutes of the first incubation, discard the contents of the wells into a discard basin. Tap the wells (face down) on a layer of absorbent paper to remove residual standard and sample. "Note: DO NOT WASH."
8. Transfer 100 µL of previously mixed contents from each mixing well to a corresponding Antibody Coated Well. Incubate for 10 minutes. Note: The mixing wells contain enough solution to run each standard and/or sample in duplicate. Cover to avoid direct light.
9. Decant the contents from the microwells into a discard basin. Wash the wells by filling with the reconstituted PBS-Tween wash buffer, then decanting the buffer into the discard basin. Repeat for a total of five washings. Tap the wells (face down) on a layer of absorbent paper to remove residual wash buffer.
10. Add 100 µL of enzyme substrate (TMB) to each well and incubate for 10 minutes. Cover to avoid direct light (TMB substrate is light sensitive).
11. Stop the reaction by adding 100 µL stop solution. The blue color will change to yellow.
12. Read the optical density (OD) of each microwell with a microplate reader at 450 nm using an air blank or a differential filter of 630 nm.

Assay Characteristics

Data from 9 consecutive standard curves gave the following results.

Aflatoxin M1 (pg/mL)	%B/B0	CV (%)
0	100	0.00
10	86	0.96
30	66	1.83
80	41	3.90
200	23	4.08
500	13	3.45



Cross reactivity

Cross reactivity of antibody to each Aflatoxin.

Aflatoxin Subtype	Cross Reactivity (%)
Aflatoxin M1	**100**
Aflatoxin M2	**<0.1**
Aflatoxin B1	**<0.1**
Aflatoxin B2	**<0.1**
Aflatoxin G1	**<0.1**
Aflatoxin G2	**<0.1**

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 standard against the Aflatoxin M_1 content of the standard. Unknowns are measured by interpolation from the standard curve.

The mean value of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the zero standard and multiplied by 100. The zero standard is thus made equal to 100 % and the absorbance values of other standards and samples are quoted in percentages of this value.

(absorbance standard or sample / absorbance zero standard) x 100 = % absorbance

The values calculated for the standards are entered in a system of coordinates on 4-parameter graph paper against the Aflatoxin M_1 concentration in pg/mL. The Aflatoxin M_1 concentration in pg/mL corresponding to the absorbance of each sample can be read from the calibration curve.

Recovery

Recovery of 50 ppt (pg/mL) spiked into milk and yogurt is as follows:

Dairy sample (pg/mL)	% Recovery
Raw milk , 50 pg/mL	79
Raw skim milk, 50 pg/mL	113
Full fat homogenized, 50 pg/mL	108
2% fat homogenized, 50 pg/mL	85
Yogurt, 50 pg/mL	112

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