Aflatoxin M1 ULTRA

ELISA Quantitative

Catalog # 961AFLM01C-ULTRA

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

This package insert must be read in its entirety before using this product.

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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 ULTRA 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.

Field of Use

Data obtained from Helica™ assays should not be used for human diagnostic or human treatment purposes. Assays are not approved by the United States Food and Drug Administration or any other U.S or non-U.S. regulatory agency for use in human diagnostics or treatment. Helica assays should not be used as the sole basis for assessing the safety of products for release to consumers. The information generated is only to be used in conjunction with the user's regular quality assurance program. Not approved for clinical diagnosis. Use for research and development, quality assurance and quality control under supervision of technically qualified persons.

Reagents Provided

1X Pouch	**Antibody coated microwell plate**		96 wells (12 eight-well strips) in a microwell holder coated with a mouse anti-aflatoxin monoclonal antibody, *Ready-To-Use*.
1X Plate	**Mixing wells**	Green	96 non-coated wells (12 eight well strips) in a microwell holder, *Ready-To-Use.*
6X Vials	**Aflatoxin M1 standards**	Black cap	8.0 mL/vial of Aflatoxin M1 at the following concentrations: 0.0, 5.0, 15.0, 50.0, 150.0, 500.0 pg/mL (ppt), *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-Use*.

Materials Required But Not Provided

- Single or multi-channel pipettor with 10, 100, 200 and 1000 µL tips
- Microtubes
- Wash bottle
- Absorbent paper towels
- Centrifuge (and tubes)
- Microplate reader with 450 nm filter
- Yogurt diluent for yogurt sample (Cat# 937YOG001)

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 15,000 g for 5 minutes to induce separation of the upper fatty layer.
4. Remove the upper fatty layer by aspiration and transfer the clean mid-plasma in a microtube for the assay.

Homogenized milk

1. Transfer 1 mL of milk in a microcentrifuge tube.
2. Centrifuge at 15,000 g for 5 minutes to induce separation of the upper fatty layer.
3. Remove the upper fatty layer by aspiration and transfer the clean mid-plasma in a microtube for the assay.

Skim milk powder

1. Reconstitute skim milk powders according to the manufacturer's instructions.
2. Transfer in a microtube for the assay.

Yogurt

1. For testing yogurt, yogurt diluent is provided separately. (Cat# 937YOG001; Please contact us for the purchase request.)
2. Weigh out 1g of yogurt in a clean tube.
3. Add 3mL of yogurt diluent into the tube.
4. Vortex the tube about 30 seconds until the mixture is homogenized.
5. Transfer in a microtube for the assay.
6. The measured value of Aflatoxin M1 from sample must be multiplied by 4 to take into account the sample dilution.

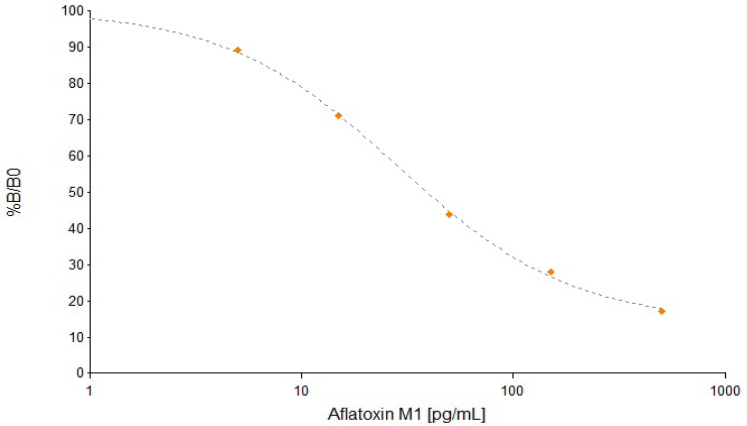
Assay Procedure

1. Bring the reagents to room temperature before use. Reconstitute the content of PBS-Tween packet with 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 twice the number of Antibody Coated Microtiter Wells in another microwell holder.
3. Aliquot 1.2 mL of standards and sample into microtubes. If running singlets, scale the volume down accordingly.
4. Using a multichannel pipettor, transfer 200 µL aliquots of standards and samples from the microtubes into Antibody Coated Wells in duplicate.
5. Incubate for 20 minutes as the first incubation.
6. 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 three washings. Tap the wells (face down) on a layer of absorbent paper.
7. Transfer 200 µL aliquots of standards and samples again. Incubate for 20 minutes as the second incubation.
8. During the second 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.
9. After the second incubation, wash the plate by repeating step 6.
10. Transfer 100 µL of the conjugate mixture from each mixing well (step 8) to a corresponding Antibody Coated Well. Incubate for 20 minutes. Cover to avoid direct light.
11. 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.
12. 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).
13. Add 100 µL of stop solution. The blue color will change to yellow.
14. 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	0.45
5	89	0.04
15	71	2.14
50	44	1.62
150	29	0.22
500	17	2.60



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

Dairy sample	Spike (ppt)	n	% Recovery	% CV
Raw milk	5	3	92	12.82
	50	3	115	1.82
	200	3	95	0.61
Homogenized milk	5	3	101	7.76
	50	3	115	2.43
	200	3	101	5.45
Skim milk powder	5	3	80	8.74
	50	3	109	4.12
	200	3	99	2.93
Yogurt	20	3	99	14.60
	50	3	112	11.40
	200	3	110	6.47

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