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Product Information

Rapid Aflatoxin M_1 ELISA Kit
for dairy products
(milk, milk powder, butter, and cheese)

Catalog Number **SE120003**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Aflatoxins are toxic metabolites that different molds like *Aspergillus flavus* and *Aspergillys parasiticus* produce. Aflatoxins are carcinogenic and can be present as contaminants in grains, nuts, cottonseed and other materials, e.g. crops, associated with animal feed or human food. In particular, four aflatoxin sub-types, B_1, B_2, G_1, and G_2 are known to occur as crop contaminants. Aflatoxin B_1 is the most toxic and frequently detected aflatoxin subtype.[1,2]

When animals such as cows consume feed that is contaminated with aflatoxin B_1, the aflatoxin B_1 is metabolically converted to aflatoxin M_1. Aflatoxin M_1 is subsequently secreted in the milk of lactating cows. Aflatoxin M_1 has stability against standard milk processing methods such as pasteurization. If aflatoxin M_1 remains present in raw milk, it may thus persist in the final products for human consumption.[3]

The Rapid Aflatoxin M_1 ELISA Kit 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. If Aflatoxin M_1 is present, it will bind to the coated antibody. Subsequently, aflatoxin bound to horseradish 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 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 450 nm (OD_{450}). The optical densities of the samples are compared to the ODs of the kit standards, and an interpolated result is determined.

The Rapid Aflatoxin M_1 Assay is intended for the quantitative detection of Aflatoxin M_1 in milk, reconstituted milk powders, butter, and cheese.

Components

1. Aflatoxin M_1 Microplate (961AFLM01MUS): 96 wells (12 × 8) well holder coated with a mouse anti-aflatoxin monoclonal antibody.
2. Aflatoxin M_1 Standards (963S6AFLM01MUS): 6 vials, 1.5 mL/vial of Aflatoxin M_1 at the following concentrations: 0.0, 100.0, 250.0, 500.0, 1,000.0, and 2,000.0 pg/mL (ppt) in stabilized skim milk.
3. Aflatoxin M_1 HRP-Conjugate (964AFLM01MUS, Green Cap): 12 mL of HRP conjugated aflatoxin in buffered solution with preservative.
4. Assay Diluent (937AD001, Brown Cap): 2 × 12 mL
5. Aflatoxin M_1-Free Skim Milk (927MK001, White Cap): 12 mL of skim milk for preparation of cheese extract.
6. TMB Substrate (916T001, Blue Cap): 12 mL of stabilized urea peroxide and 3,3′,5,5′-tetramethyl-benzidine (TMB).
7. Stop Solution (946P001, Red Cap): 12 mL of Acidic Solution.
8. PBST Wash Buffer Powder (915X001): 1 packet of PBS with 0.05% TWEEN® 20. Bring to 1 liter with distilled water and store refrigerated.
9. Dilution Wells (Green): 1 plate, 96 wells (12 × 8 well strips), non-coated, in microwell holder. The wells are color-coded green.

Reagents and Equipment Required but Not Provided

1. Microplate reader capable of measuring absorbance at 450 nm
2. Single or multichannel pipettor with 100 µL and 200 µL tips
3. Absorbent paper towels
4. Graph paper, or computer and software for ELISA data analysis
5. Glass tubes
6. Centrifuge
7. Balance
8. Methanol

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices. Consider all materials, containers, and devices that are exposed to sample or standards to be contaminated with aflatoxin M_1. Wear protective gloves and safety glasses when using this kit.

Storage/Stability

Store reagents at 2–8 °C, and do not use beyond expiration date(s). Never freeze the kit components.

Bring all reagents to room temperature (19–25 °C) before use.

HRP-labeled conjugate and TMB Substrate are photosensitive and are packaged in protective opaque bottles. Store in the dark and return to storage after use.

Do not return unused reagents back to their original bottles.

Before doing the assay, prepare a waste container as a receptacle for kit waste. Eject contaminated pipette tips and all other related materials into this container. Following completion of the assay, treat the container with sufficient 5-6% sodium hypochlorite (NaOCl) to saturate the container's contents, about $1/10^{th}$ the volume of the container. 5-6% NaOCl will denature the mycotoxins and neutralize the waste, which renders the waste safe for disposal. Invert the container several times to coat all waste thoroughly.

(In case of an accidental toxin spill, treat the spill surface with 5-6% NaOCl for a minimum of 10 minutes, and then with 5% aqueous acetone. Wipe dry with absorbent paper towels.)

Preparation Instructions

Extraction Procedure / Sample Preparation

Raw Milk

1. The standards are presented in homogenized skim milk. 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. At this point, centrifugation 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. Then centrifuge at 2,000 × *g* for 5 minutes to induce separation of the upper fatty layer.
4. Remove the upper fatty layer by aspiration. Use the lower plasma in the assay.
5. The sample is now ready. The standards do not require pre-dilution before use.

Homogenized Milk

1. Homogenized skim milk should be used directly in the assay.
2. Due to the stabilization of the fat globules induced by the homogenizing process, they are difficult to eliminate, even by high-speed centrifugation, in order to create a plasma from homogenized fatty milk. Therefore, use homogenized fatty milk directly in the assay.

Milk Powder

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

Cheese

1. 1 g of finely grated or otherwise macerated cheese is mixed with 5 mL of absolute methanol in a capped tube and mixed for 5 minutes. The tube is clarified by centrifugation (5,000 × *g* for 5 minutes), and the supernatant is removed.
2. 0.5 mL of this supernatant is transferred to a glass tube and the methanol evaporated by a stream of air (recovery is better with nitrogen gas). This procedure results in the deposition of a semi-solid viscous material on the inside of the tube.
3. Add 0.5 mL of the provided blank skim milk (927MK001) to the tube and vortex vigorously for 1 minute. Allow the tube to stand for a further 5 minutes. Use this milk extract in the assay.

Butter

1. Prepare 50% methanol extraction buffer by addition of equal volumes of water and pure methanol (e.g. 2.5 mL of methanol plus 2.5 mL of deionized water).
2. Weigh 5 g of butter into a vessel.
3. Add 5 mL of extraction buffer.
4. Place the sample in a 37 °C water bath to melt the butter. When completely melted, mix the sample thoroughly for 1 minute.
5. Centrifuge the sample for 5 minutes at 3,500 rpm. A fatty layer may form and solidify at the top of the extract. Use the sample underneath the fatty layer for the assay.

Procedure

1. Reconstitute the PBST buffer into 1 L of distilled water. The remaining content packets may be washed out with a gentle stream of distilled water. Store the reconstituted buffer 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 microwells in a separate microwell holder.
3. Return unused wells to the pouch and reseal with tape to avoid moisture entry. Retain the well holder for future use.
4. Dispense 200 µL of the Assay Diluent into each Dilution Well.
5. Using a fresh pipette tip for each, dispense 100 µL of standards and samples into the appropriate wells and mix by aspirating three times.
6. Using a multichannel pipette, transfer 100 µL of the mixture to the appropriate assay well and incubate at ambient temperature for 10 minutes.
   Note: The Dilution Wells contain enough for 100 µL to be run in duplicate.
7. Place sufficient conjugate (120 µL per standard/ sample) in a trough. With a multichannel pipette, add 100 µL of conjugate to the wells already containing standard/sample. Do not empty or wash the wells before adding the conjugate. The force of the addition of the second 100 µL to the first 100 µL causes sufficient mixing.
8. Continue the incubation for a further 30 minutes.
9. Discard the contents of the wells into an appropriate receptacle. Wash the wells by filling with PBST buffer. Discard the washings (e.g. by decanting), and repeat for a total of three washes.
10. Add 100 µL of enzyme substrate (TMB) to each well and incubate for 10 minutes. Cover to avoid direct light.
11. Stop the reaction by adding 100 µL of stop solution. The blue color will change to yellow.
12. Read and record the optical density (OD) of each microwell at 450 nm, using either an air blank or a differential filter of 630 nm.
13. With the zero standard set as 100% binding (Bo), calculate the % binding (%B) for each standard and sample as a percentage of the zero binding (%B/Bo).

Results

Construct a standard 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%. The absorbance values of other standards and samples are quoted in percentages of this value.

The values calculated for the standards are entered in a system of coordinates on semilogarithmic 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 standard curve.

In order to obtain the aflatoxin M_1 concentration (pg/mL) of a sample, the concentration obtained from the calibration curve must be further multiplied by the corresponding dilution factor. This is 1 for milk and butter samples, and 5 for cheese samples.

References

1. Klich, M.A., Environmental and developmental factors influencing by *Aspergillus flavus* and *Aspergillus parasiticus*. *Mycoscience,* **48(2)**, 71-80 (2007).
2. Williams, J.H. *et al.*, Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. *Am. J. Clin. Nutr.*, **80(5)**, 1106-1122 (2004).
3. Ismail, A. *et al.*, Aflatoxin M1: Prevalence and decontamination strategies in milk and milk products. *Crit. Rev. Microbiol.*, **42(3)**, 418-427 (2016).

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