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Product Information

Aflatoxin M_1 ELISA Kit for Urine

Catalog Number **SE120005**
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 consume food that is contaminated with aflatoxin B_1, the aflatoxin B_1 is metabolically converted to aflatoxin M_1, in a hydroxylation reaction.[3,4] Aflatoxin M_1 is excreted in urine.[5] The conversion rate of aflatoxin B_1 to aflatoxin M_1 has been estimated at ~2%.[6,7]

The Aflatoxin M_1 ELISA Kit for Urine is a direct ELISA kit in which an antibody with high affinity for aflatoxin M_1 is coated onto polystyrene microwells. After initial dilution with distilled water, the urine sample is mixed with assay buffer and added to the well. If aflatoxin M_1 is present in the urine, 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 present in the sample or standard. After this incubation period, the contents of the wells are decanted and washed. 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 in the standard or the sample. 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 450 nm (OD_{450}). The optical densities of the samples are compared to the OD's of the kit standards, and a result is determined by interpolation from the standard curve.

This Aflatoxin M_1 assay kit is for the quantitative determination of aflatoxin M_1 in urine. Different estimates of the limit of detection (LOD) of the kit have included 0.2 ng aflatoxin M_1 per mL urine,[8] and 30 pg (0.03 ng) of aflatoxin M_1 per mL of urine.[9] The limit of quantitation (LOQ) of the kit has been estimated to be 0.4 ng aflatoxin M_1 per mL urine.[8]

Components

1. Aflatoxin M_1 Microplate (991AFLM01U): 96 wells (12 × 8) in a microwell holder coated with a mouse anti-aflatoxin monoclonal antibody.
2. Aflatoxin M_1 Standard (993S1AFLM01U): 6 vials, 1.5 mL/vial of Aflatoxin M_1 at the following concentrations: 0.0, 0.15, 0.40, 0.80, 1.50, and 4.00 ng/mL in stabilized normal human urine
3. Aflatoxin M_1 HRP-Conjugate (994MAFLM01U): 12 mL of HRP-conjugated aflatoxin in buffered solution with preservative
4. Assay Diluent (937AD001): 2 × 12 mL of propriety assay buffer
5. TMB Substrate (916T001): 12 mL of stabilized urea peroxide and 3,3′,5,5′-tetramethylbenzidine (TMB)
6. Stop Solution - 946P001: 12 mL of Acidic Solution
7. PBST Wash Buffer Powder (915X001): 1 packet of PBS with 0.05% TWEEN® 20. Bring to 1 liter with distilled water and store refrigerated.
8. Mixing Wells (Red): 1 plate, 96 non-coated wells (12 eight-well strips) in a microwell holder

Reagents and Equipment Required but Not Provided.

1. Microplate reader capable of measuring absorbance at 450 nm
2. Precision pipettes to deliver 100-200 µL volumes
3. Distilled or deionized water
4. Absorbent paper towels
5. Graph paper or computer and software for ELISA data analysis
6. Glass tubes
7. Timer

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.

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

HRP-labelled conjugate and TMB Substrate are photosensitive and are packaged in protective opaque bottles. Store in the dark and return to storage. Do not return unused reagents back into 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.)

Storage/Stability

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

Procedure

1. Reconstitute the PBS-T Wash Buffer powder to 1 L. The packet contents may be washed out with a gentle stream of distilled water if needed. Refrigerate the reconstituted PBS-T Wash Buffer when not in use.
2. Remove any debris or precipitate from the urine sample by filtration or centrifugation.
3. Dilute an aliquot of both the urine standards and samples 20-fold with distilled water (e.g., 50 μL plus 950 μL of distilled water).
4. Place one mixing well in a microwell holder for each standard and sample to be tested. Place an equal number of antibody-coated microwells in another microwell holder.
5. Dispense 200 μL of the assay buffer into each mixing well.
6. Using a new pipette tip for each, add 100 μL of each diluted standard and sample to the appropriate mixing well containing the assay buffer. Mix by priming pipettor at least 3 times.
7. Using a new pipette tip for each, transfer 100 μL of contents from each mixing well to a corresponding antibody-coated microwell. Incubate at room temperature for 1 hour. The mixing wells contain enough solution to run each standard and/or sample in duplicate if so desired.
8. Decant the contents from the microwells into a discard basin. Wash the microwells by filling each with PBST wash buffer, then decanting the wash into a discard basin. Repeat wash for a total of 3 washes.
9. Tap the microwells (face down) on a layer of absorbent towels to remove residual wash buffer.
10. Add 100 μL of conjugate to each antibody-coated well. Incubate at ambient temperature for 15 minutes.
11. Repeat step 8 for the washing procedure.
12. Measure the required volume of Substrate Reagent (1 mL/strip or 120 μL/well) and place in a separate container. Add 100 μL to each microwell. Incubate covered from light at room temperature for 15 minutes. Cover to avoid direct light.
13. Measure the required volume of Stop Solution (1 mL/strip or 120 μL/well) and place in a separate container. Add 100 μL in the same sequence and at the same pace as the Substrate was added.
14. Read and record the optical density (OD) of each microwell with a plate reader using a 450 nm filter within 15 minutes of adding stop solution.
    Note: If more than two strips are used in an assay, a multichannel pipettor is recommended, to mitigate "beginning to end" variation.

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 (0.0) standard against the aflatoxin content of the standard. Unknowns are measured by interpolation from the standard curve.

If a sample gives an OD less than the highest standard, it should be further diluted in distilled water and retested. The extra dilution should be taken into account when calculating the result.

Due to the nature of inhibition immunoassays, values derived by extrapolation outside of the measured highest and lowest standards are likely to be erroneous**.**

Product Profile

Recovery

Urine samples were spiked with various levels of aflatoxin M_1 in separate experiments, and the % recoveries were measured. Mean recovery values, from 18 samples, are given below:

Sample Type	Average % recovery	Recovery range %
Urine (0.5 ng/mL)	96.4	78-111
Urine (2.0 ng/mL)	96.5	73-109

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