Product Information

Aflatoxin M₁ ELISA Kit for Urine

Catalog Number SE120005
Storage Temperature 2-8 °C

TECHNICAL BULLETIN

Product Description
The Sigma-Aldrich® Aflatoxin M₁ ELISA (Enzyme-Linked Immunosorbent Assay) Kit for Urine is a direct enzyme linked immunosorbent assay in which an antibody with high affinity for aflatoxin M₁ is coated onto polystyrene microwells. After an initial dilution with distilled water, the urine sample is mixed with assay buffer and added to the well. If aflatoxin M₁ is present in the urine 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 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 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 (OD₄₅₀). 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.

Intended Use
The Aflatoxin M₁ in urine assay is an enzyme-linked immunosorbent assay for the quantitative determination of aflatoxin in urine at levels which should be helpful in monitoring populations at risk for acute or chronic aflatoxicosis. The assay has not yet been approved by FDA for diagnostic purposes.

Components
1. Aflatoxin M₁ Microplate - 991AFLM01U: 96 wells (12×8) well holder coated with a mouse anti-aflatoxin monoclonal antibody.
2. Aflatoxin M₁ Standard - 993S1AFLM01U: 6 vials, 1.5 mL/vial of Aflatoxin M₁ 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 M₁ HRP-Conjugate - 994MAFLM01U, 12 mL of HRP conjugated aflatoxin in buffered solution with preservative.
4. Assay Diluent - 937AD001: 2 × 12 mL propriety assay buffer.
5. TMB Substrate - 916T001: 12 ml of stabilized urea peroxide and 3,3’,5,5’-tetramethylbenzidine (TMB).
7. PBST Wash Buffer Powder - 915X001: 1 packet of PBST. Bring to 1 liter with distilled water and store refrigerated.
8. Dilution 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.

Precautions and Disclaimer
1. This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.
2. Bring all reagents to room temperature (19-27 °C) before use.
3. Consider all materials, containers and devices that are exposed to sample or standards to be contaminated with aflatoxin M₁. Wear protective gloves and safety glasses when using this kit.
4. HRP-labelled conjugate and TMB Substrate are photosensitive and are packaged in a protective opaque bottle. Store in the dark and return to storage.
Storage/Stability
Store reagents at 2-8 °C, and do not use beyond expiration date(s). Never freeze kit components.

Assay Procedure
1. Bring all the reagents to room temperature before 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 1:20 with distilled water e.g., 50 μL plus 950 μL 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 Microtiter Wells 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 Microtiter Well. 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 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 water.
10. Add 100 μL of conjugate to each antibody coated well and incubate at ambient temperature for 15 minutes.
11. Repeat step 8 for 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 the optical density (OD) of each microwell with a microtiter plate reader using a 450 nm filter within 15 minutes of adding stop solution. Record the optical density (OD) of each microwell.

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 re-tested. 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 M1 in separate experiments and the % recoveries measured. Mean recovery is given below:

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Average % Recovery</th>
<th>Recovery range %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>96.4</td>
<td>78-111</td>
</tr>
<tr>
<td></td>
<td>96.5</td>
<td>73-109</td>
</tr>
</tbody>
</table>

References

MJM, GA,PHC 08/14-1