Aflatoxin M1 (AFM1) ELISA Kit

Catalog No.: abx364886

Size: 96T

Sensitivity: 0.05 ng/ml (ppb)

Detection Limit: Milk – 0.1 ng/ml; Milk powder – 0.15 ng/ml; Urine – 0.5 ng/ml

Sample Recovery Rate: Milk – 85% ± 15%; Milk powder, Urine – 80% ± 15%

Cross-Reactivity: Aflatoxin M1 – 100%

Storage: Store all components at 4°C.

Application: For quantitative detection of AFM1 in urine, liquid milk, and milk powder.

Introduction: Aflatoxin M1 is an aflatoxin produced by Aspergillus flavus and A.parasiticus. It is a potent carcinogen, especially in smaller mammals including rats. Aflatoxin M1 is a metabolite of aflatoxin B1 in humans and animals and can be found in milk and dairy products. Aflatoxin M1 is also associated with liver damage and cancer resulting from AFM1-mediated activation of P450 to the epoxide which alkylates DNA.

Principle of the Assay

This kit is based on competitive enzyme-linked immuno-sorbent assay technology. An antigen is pre-coated onto a 96-well plate. Controls, test samples, HRP-conjugated reagent and biotin-conjugated reagent are added to the wells and incubated. A competitive inhibition reaction takes place between the pre-coated AFM1 and the AFM1 in the sample with the biotin-labelled antibody. Unbound conjugates are removed using wash buffer at each stage. TMB substrate is used to quantify the HRP enzymatic reaction. After TMB substrate is added, only wells that contain sufficient AFM1 will produce a blue-colored product, which then changes to yellow after adding the acidic stop solution. The intensity of the yellow color is inversely proportional to the AFM1 concentration in the sample. The OD is measured spectrophotometrically at 450 nm in a microplate reader, from which the concentration of AFM1 can be calculated.

Kit components

1. One pre-coated 96 well plate
2. Standard: 1 ml each (0 ng/ml, 0.05 ng/ml, 0.15 ng/ml, 0.45 ng/ml, 1.35 ng/ml, 4.05 ng/ml).
3. Detection Reagent A: 5.5 ml
4. Detection Reagent B: 5.5 ml
5. TMB Substrate A: 6 ml
6. TMB Substrate B: 6 ml
7. Stop Solution: 6 ml
8. Wash Buffer (20X): 40 ml
9. Reconstitution Buffer (2X): 50 ml
10. Plate Sealer: 3
11. Hermetic Bag: 1

Material Required But Not Provided

1. Microplate reader (wavelength: 450 nm)
2. High-precision pipette and sterile pipette tips
3. Centrifuge or microfuge tubes
4. Distilled or deionized water
5. Absorbent filter papers
6. 100 ml and 1 L graduated cylinders
7. Nitrogen evaporator or water bath
8. Balance (0.01 g)
9. Orbital shaker and vortexer
10. Centrifuge
11. Acetonitrile (100 %)

Protocol

A. Preparation of sample and reagents

1. 1X Reconstitution Buffer

Dilute the concentrated Reconstitution Buffer 2-fold (1/2) with deionized water (e.g. add 25 ml of concentrated reconstitution solution into 25 ml of distilled water).

2. 1X Wash Buffer

Dilute the concentrated Wash Buffer 20-fold (1/20) with distilled water (e.g. add 40 ml of concentrated wash buffer into 760 ml of distilled water).

3. 84% Acetonitrile Solution

Dilute neat Acetonitrile with distilled water to a ratio of 84:16 (e.g. add 8.4 ml of neat acetonitrile into 1.6 ml of distilled water).

4. Samples

Aflatoxin M1 can be unevenly distributed in samples. It is recommended to take several samples from each subject where possible.

- **Liquid Milk:** Add 1 ml of liquid milk to a 50 ml centrifuge tube. Add 4 ml of neat (100%) acetonitrile and vortex for 5 minutes. Centrifuge at 4000 × g for 10 minutes at room temperature. Take 2.5 ml of supernatant and add to another centrifuge tube. Dry using a nitrogen evaporator or water bath at 50°C. Dissolve the residue with 1 ml of 1X Reconstitution Buffer. Vortex to mix fully. Take 50 µl of the sample for detection and analysis.
  Note: the sample dilution factor is 2 and the minimum detection dose is 0.1 ng/ml.

- **Milk Powder:** Add 5 g of milk powder to a 50 ml centrifuge tube. Add 20 ml of 84% Acetonitrile Solution and vortex for 5 minutes. Centrifuge at 4000 × g for 10 minutes at room temperature. Take 1 ml of clear liquid and add to another centrifuge tube. Dry using a nitrogen evaporator or water bath at 50°C. Dissolve the residue with 0.75 ml of 1X Reconstitution Buffer. Vortex to mix fully. Take 50 µl of the sample for detection and analysis.
  Note: the sample dilution factor is 3 and the minimum detection dose is 0.15 ng/ml.

- **Urine:** Centrifuge cloudy samples at 4000 × g for 5 minutes at room temperature to produce clear samples. Add 0.1 ml of clear sample to 0.9 ml of 1X Reconstitution Buffer and mix thoroughly. Take 50 µl of the sample for detection and analysis.
  Note: the sample dilution factor is 10 and the minimum detection dose is 0.5 ng/ml.

B. Assay Procedure

Bring all samples and reagents to room temperature (25°C) prior to use. A room temperature or reagent temperature lower than 25°C may result in lower OD value readings.

1. Any strips that are not being used should be kept dry and stored at 4°C. Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate.
2. Add 50 µl of the prepared standards solutions into the standard wells.
3. Add 50 µl of the 0 ng/ml standard into the control (zero) wells.
4. Add 50 µl of appropriately prepared samples into the test sample wells.
5. Immediately add 50 µl of Detection Reagent B into each well, and then add 50 µl of Detection Reagent A into each well. Add the solution at the bottom of each well without touching the side wall.
6. Cover the plate with the plate sealer. Gently tap the plate to mix thoroughly. Incubate at 25°C for 30 minutes in the dark.
7. Remove the cover and discard the solution. Wash the plate 5 times with 1X Wash Buffer. *Fill each well completely with Wash buffer (300 µl) using a multi-channel Pipette or automated washer (30 seconds per wash). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the*

plate and blot it against clean absorbent paper towels.

8. Add 50 µl of Substrate Reagent A to each well, and then 50 µl of Substrate Reagent B into each well. Cover the plate and gently tap the plate to mix thoroughly. Incubate at 25°C in the dark for 15 minutes. The incubation time is for reference only, the optimal time should be determined by end user.
9. Add 50 µl of Stop Solution into each well. There should be a color change to yellow. Gently tap the plate to ensure thorough mixing. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
10. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm in a microplate reader within 10 min of adding the stop solution.
11. OPTIONAL: Measure the absorbance at 630 nm and subtract these values from the 450 nm values for analysis.
12. OPTIONAL: Calculate the absorbance percentage by dividing each OD value by the OD value of the 0 ng/ml standard, and multiplying by 100 to give a percentage.

    To calculate the absorbance, use the following equation: Absorbance (%) = A / A_0 × 100% (where A: average absorbance of standard or sample, A_0: average absorbance of 0 ng/ml of the standard). The standard curve can be plotted as the absorbance of each standard solution vs. the respective concentration of the standard solution. The AFM1 concentration of the samples can be determined from the standard curve.

    Note: If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.

C. Notes and Precautions

- Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
- Do not leave the wells uncovered for extended periods between incubations. The addition of reagents for each step should not
- exceed 10 mins.
- Ensure that the plate is properly sealed or covered during the incubation steps, and that the time and temperature are controlled.
- Do not reuse pipette tips and tubes.
- Do not use expired components, or components from a different kit.
- The TMB substrate reagent solutions should be used under sterile conditions, and light exposure should be minimized. Unused substrate should be colorless, or a very light yellow in appearance. Do not discard any residual solution back into the vial. If the stock substrate reagent solutions turn a blue color and/or the OD value of the 0 ng/ml standard (blank) is < 0.5, it is likely that the substrate reagent solutions have been contaminated or exposed to excessive light.
- Please note that this kit is optimized for detection of native samples, rather than recombinant proteins or synthetic chemicals. We are unable to guarantee detection of recombinant proteins, as they may have different sequences or tertiary structures to the native protein.
- This kit is for qualitative detection of AFM1 in urine, liquid milk, and milk powder samples. Suitability for other sample types would need to be determined by the end user.