8th Edition, revised in February, 2018

AFM1 (Aflatoxin M1) ELISA Kit

Catalog No: E-TO-E012

96T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) Fax: 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

This kit uses Indirect-Competitive-ELISA as the method. It can detect Aflatoxin M1 (AFM1) in urine samples. This kit is composed of ELISA Microplate, HRP conjugate, antibody, standard and other supplementary reagents. The ELISA Microplate provided in this kit has been pre-coated with AFM1. During the reaction, AFM1 in the samples or standard competes with AFM1 on the solid phase supporter for sites of AFM1 antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well, and TMB substrate is for color development. There is a negative correlation between the OD value of samples and the concentration of AFM1. The concentration of AFM1 in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Sensitivity: 0.05 ppb (ng/mL)
Reaction mode: 25℃, 30 min～15 min
Detection limit: Urine---1.5 ppb,
Cross-reactivity: Aflatoxin M1 (AFM1) ---100%
Sample recovery rate: Urine ---85%±15%

Kits components

Item	Specifications
ELISA Microplate	96 wells
Standard Liquid	1 mL each (0 ppb, 0.05 ppb, 0.15 ppb, 0.45 ppb, 1.35 ppb, 4.05ppb)
HRP Conjugate	5.5 mL
Antibody Working Solution	5.5 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
20×Concentrated Wash Buffer	40 mL
2×Reconstitution Buffer	40 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Other supplies required

Instruments: Microplate reader, Printer, Homogenizer, Oscillators, Centrifuge, Graduated pipette, Balance (sensibility 0.01 g).

High-precision transferpettor: Single channel (20-200 μL, 100-1000 μL), Multichannel (300 μL).

Reagents: Acetonitrile, Deionized Water.

Experimental preparation

Bring all reagents and samples to room temperature before use.

Open the microplate reader in advance, preheat the instrument, and set the testing parameters.

1. **Sample pretreatment Notice:** experimental apparatus should be clean, and use disposable pipette tips to avoid cross-contamination during the experiment.

2. **Solution preparation**

Solution 1: Reconstitution Buffer

Dilute the 2×Reconstitution Buffer with deionized water. (2×Reconstitution Buffer (volume): Deionized water (volume) = 1:1).

Solution 2: Wash Buffer

Dilute the 20×Concentrated Wash Buffer with deionized water. (20×Concentrated Wash Buffer (V): Deionized water (V) =1:19).

3. Sample pretreatment procedure

3.1 Pretreatment of urine:

(1) Take 100 μL of urine and all 900 μL of reconstitution buffer into EP tube, oscillate for 1min;

(2) Take 50 μL of sample for analysis.

Note: Sample dilution factor: 10, minimum detection dose: 1.5 ppb

Assay procedure

Centrifuge the sample again after thawing before the assay. Bring all reagents to room temperature for 30 min before use. **All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.**

1. **Number:** number the sample and standard in order (multiple wells), and keep a record of standard wells and sample wells.
2. **Add sample:** add 50 μL of Standard or Sample per well, then add 50 μL of HRP conjugate to each well, then add 50 μL of antibody working solution, cover the plate sealer, oscillate for 5 s gently to mix thoroughly, incubate for 30 min at 25℃.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 300 μL of wash buffer to each well and wash. Repeat wash procedure for 5 times, 30 s intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **Color Development:** add 50 μL of substrate solution A to each well, and then add 50 μL of substrate solution B. Gently oscillate for 5 s to mix thoroughly. Incubate shading light for 15 min at 25℃. (If the blue color is too shallow, can extend the incubation time properly).
5. **Stop reaction:** add 50 μL of stop solution to each well, oscillate gently to mix thoroughly.
6. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm with a microplate reader (the 450/630 nm double wavelength is recommended). This step should be finished in 10min after stop reaction.

Result analysis

1. **Absorbance (%) =A/A_0 ×100%**

   A: Average absorbance of standard or sample

   A_0: Average absorbance of 0 ppb Standard

2. **Drawing and calculation of standard curve**

   Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot. Add average absorbance value of sample to standard curve to get corresponding concentration. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.

   For this kit, it is more convenient to use professional analysis software for accurate and fast analysis on a large number of samples.

Notes

1. Overall OD value will be lower when reagents is not brought to room temperature before use or room temperature is below 25℃.
2. During the washing procedure, if the wells turn dry, it will lead to bad linear standard curve and poor repeatability, move on to the next step immediately after wash.
3. Mix thoroughly and wash the plate completely. The consistency of wash procedure can strongly affect the reproducibility of this ELISA kit.
4. ELISA Microplate should be covered by plate sealer. Avoid the reagents to strong light.
5. Do not use expired kit and reagents of different batches.
6. TMB should be abandoned if it turns color. When OD value of standard(concentration: 0)＜0.5 unit($A_{450\ nm}$＜0.5), it indicates reagent is deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.

Storage and valid period

Storage: Store at 2-8℃. Avoid freeze / thaw cycles.

Valid Period: 1 year, expiration date is on the packing box.