Elabscience®

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AFM1 (Aflatoxin M1) ELISA Kit

Catalog No: E-TO-E007

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) 240-252-7376(USA)
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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 AflatoxinM1 (AFM1) in samples, such as liquid milk and milk powder samples. This kit is composed of Micro ELISA Plate, HRP conjugate, antibody, standard and other supplementary reagents. The micro-plate 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 AFM1antibody. 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 ofAFM1. 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: Liquid milk---0.1 ppb; Milk powder---0.15 ppb

Cross-reactivity: AflatoxinM1 (AFM1) ---100%

Sample recovery rate: Liquid milk---85%±15%, Milk powder---80%±15%

Kits components

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

Other supplies required

Instrument: Micro-plate reader, Printer, Homogenizer, Nitrogen Evaporators, 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 micro-plate reader in advance, preheat the instrument, and set the testing parameters.

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

2. Solution preparation

Solution 1: (Reconstitution fluid): for sample re-dissolution. 2× Reconstitution fluid (volume): Deionized water(volume)=1:1. Store at 4℃, valid for 1 month.

Solution 2: (sample extracting solution): 84% acetonitrile. Acetonitrile (volume): deionized water (volume) =84:16.

Solution 3: (1× wash buffer): 20×Concentrated Wash Buffer (volume): deionized water (volume) = 1:19.

3. Sample pretreatment procedure

3.1. Pretreatment of liquid milk:

(1) Take 1 mL liquid milk into 50 mL EP tube, add 4 mL of acetonitrile, oscillate for 5 min, centrifuge at 4000 r/min for 10 min at room temperature;
(2) Take 2.5 mL of supernatant, dry with nitrogen evaporators at 50℃ or with water bath;
(3) Add 1 mL of Reconstitution fluid, oscillate and mix;
(4) Take 50 μL for detection and analysis.

Note: Sample dilution factor: 2, minimum detection dose: 0.1 ppb

3.2. Pretreatment of milk powder:

(1) Weight 5 g of milk powder into 50 mL EP tube, add 20 mL of sample extracting solution, oscillate for 5 min, filter or centrifuge at 4000 r/min for 10 min at room temperature.
(2) Take 1 mL of filtrate or supernatant, dry with nitrogen evaporators at 50℃ or with water bath.
(3) Add 750 μL of Reconstitution fluid, oscillate and mix.
(4) Take 50 μL for detection and analysis.

Note: Sample dilution factor: 3, minimum detection dose: 0.15 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.** Dilute 40mL of concentrated wash buffer into 800 mL wash buffer with deionized or distilled water.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells.
2. **Add sample:** add 50 μL of Standard, Blank, or Sample per well, and add 50 μL of HRP conjugate to each well, then add 50 μL of antibody working solution, cover the plate with sealer we provided, oscillate for 5 s gently to mix thoroughly, incubate for 30 min at 25℃.
3. **Wash:** uncover the sealer carefully, remove the liquid of each well. Immediately add 250 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. **HRP Conjugate:** add 100 μL of HRP conjugate to each well, incubate for 30 min at 37℃.
5. **Wash:** repeat step 3.
6. **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℃.
7. **Stop reaction:** add 50 μL of stop solution to each well, oscillate gently to mix thoroughly.
8. **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 10 min after stop reaction.

Result analysis

1. **Absorbance (%)=$A/A_0 \times 100\%$**

   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. Micro ELISA plate 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, production date is on the packing box.