8th Edition, revised in February, 2018

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) Fax: 240-252-7376(USA)
Email: techsupport@elabscience.com
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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 Aflatoxin M1 (AFM1) in samples, such as liquid milk and powdered milk. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody, standard and other supplementary reagents. The microplate provided in this kit has been pre-coated with coupled antigen. During the reaction, AFM1 in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti-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: Liquid milk---0.1 ppb, Milk powder ---0.15 ppb
Cross-reactivity: Aflatoxin M1 (AFM1) ---100%
Sample recovery rate: Liquid milk ---85%±15%, Milk powder ---80%±15%

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1 mL each (0 ppb, 0.05 ppb, 0.15 ppb, 0.45 ppb, 1.35 ppb, 4.05 ppb)
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	50 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Other supplies required

Instrument: Microplate reader, Printer, Homogenizer, Nitrogen Evaporators/Water bath, 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 Buffer

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

Solution 2: sample extracting solution (84% acetonitrile solution)

Acetonitrile (volume): deionized water (volume) =84:16.

Solution 3: Wash Buffer

Dilute the 20×Concentrated Wash Buffer with deionized water. (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 centrifuge 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/water bath at 50℃.
3. Dissolve the residual with 1 mL of reconstitution buffer, oscillate and mix fully.
4. Take 50 μL for analysis.

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

3.2. Pretreatment of milk powder/dairy product:

1. Weight 5 g of milk powder into 50 mL centrifuge tube, add 20 mL of sample extracting solution, oscillate for 5 min, centrifuge at 4000 r/min for 10 min at room temperature.
2. Take 1 mL filtrate or clear liquid, dry with nitrogen evaporators/water bath at 50℃.
3. Dissolve the residual with 750 μL of reconstitution buffer, oscillate and mix fully.
4. Take 50 μL for analysis.

Note: Sample dilution factor: 3, minimum detection dose: 0.15 ppb

Assay procedure

Restore all reagents and samples to room temperature 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 well), and keep a record of standard wells and sample wells. **Standard and Samples need test in duplicate.**
2. **Add sample:** add 50 μL of **standard/sample** to standard/sample well, and add 50 μL of **HRP conjugate** to each well, then add 50 μL of **antibody working solution**, cover the plate with sealer, 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 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 with shading light for 15 min at 25℃ (The reaction time can be extended according to the actual color change).
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 (reference wavelength 630 nm) with a microplate reader. This step should be finished in 10 min 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. Micro ELISA plate should be covered by plate sealer. Avoid the reagents to strong light.
5. **Do not use expired kit, reagents of different batches and reagents that do not belong to this kit**.
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.
8. As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.
9. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
10. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
11. The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS, etc. can be used for quantitative confirmation.

Storage and valid period

Store at 2~8℃ for 1 year. Avoid freeze.

Please store the opened kit at 2~8℃, protect from light and moisture. The valid period is 2 months.

Expiry date: expiration date is on the packing box.