8th Edition, revised in February, 2018

AFM1 (Aflatoxin M1) ELISA Kit

Catalog No: E-TO-E018

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 Competitive-ELISA as the method. It can detect Aflatoxin M1 (AFM1) in samples, such as raw milk, pure milk, reconstituted milk, and yogurt. 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 Microtiter 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: 0.005 ppb (ng/mL)

Reaction mode: 25℃, 30 min~20 min

Detection limit: Raw milk/ pure milk /reconstituted milk/yogurt---0.025ppb

Cross-reactivity: Aflatoxin M1 ---100%,
Aflatoxin M2 ---< 1%, Aflatoxin B1---40%, Aflatoxin B2---3%, Aflatoxin G1---20%, Aflatoxin G2---2%.

Sample recovery rate: 100%±30%.

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1.5mL each (0ppb, 0.005ppb, 0.01ppb, 0.03ppb, 0.09ppb, 0.27ppb)
HRP Conjugate	12ml
Antibody Working Solution	7mL
Yogurt Sample Dilution	10ml
Sample Dilution	45mL
Substrate Reagent A	7mL
Substrate Reagent B	7mL
Stop Solution	7mL
20×Concentrated Wash Dilution	25mL
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

Instruments: Microplate reader, Homogenizer, Vortex Oscillators, Centrifuge, Balance (sensibility 0.01 g).

Micropipettor: Single-channel (20-200 μL, 100-1000 μL).

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 the pipette should be disposable to avoid cross-contamination during the experiment.

2. **Solution preparation**

Solution 1: Wash Dilution Buffer

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

3. **Sample pretreatment procedure**

3.1 Pretreatment of raw milk/pure milk/reconstituted milk

(1) **reconstituted milk(powder):** Weigh 1 ± 0.05g of reconstituted milk (powder) with 5mL of deionized water, oscillate for 2 min, mix fully.

(2) Take 50 μL for analysis.

(3) **raw milk/pure milk/reconstituted milk(liquid):** Take 50 μL for analysis

Note: Sample dilution factor: 1, minimum detection dose: 0.025 ppb.

3.2 Pretreatment of yogurt

(1) Weigh 1±0.01g of sample with 5mL of deionized water, add 0.1 mL of yogurt Sample Dilution and 0.9 mL of Sample Dilution, oscillate for 1 min, mix fully.

(2) Centrifuge at 4000 r/min for 5 min at room temperature.

(3) Take 50 μL for analysis.

Note: Sample dilution factor: 2, minimum detection dose: 0.025 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 50μL of **standard or sample** per well, add 50μL **antibody working solution** to each well, cover the plate with sealer. Oscillate for 10 sec gently to mix thoroughly, incubate with shading light for 60 min at 25℃.
3. **Wash:** uncover the sealer carefully, remove the liquid of each well. Immediately add 260 μL of wash **dilution buffer**. Repeat wash procedure for 4 times, 30 sec 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. **Add HRP conjugate:** add 100 μL of **HRP Conjugate** to each well, incubate for 30 min at 25℃ in the dark.
5. **Wash:** repeat step 3
6. **Color Development:** add 50μL of **substrate reagent A** to each well, and then add 50μL of **substrate reagent B**. Gently oscillate for 10 sec to mix thoroughly. Incubate with shading light for 15 min at 25℃. (The reaction time can be extended according to the actual color change).
7. **Stop reaction:** add 50 μL of **stop solution** to each well. Gently oscillate for 10s to mix thoroughly
8. **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 5 min after stop reaction.

Result analysis

1. **Absorbance% =A/A_0×100%**
   A: Average absorbance of standard solution or sample
   A_0: Average absorbance of 0 ppb Standard solution
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 the average absorbance value 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. The overall OD value will be lower when reagents have not been brought to room temperature before use or room temperature is below 25℃.
2. If the wells turn dry during the washing procedure, it will lead to bad linear standard curve and poor repeatability. Operate 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 Microtiter 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 (Substrate Reagent A or Substrate Reagent B) should be abandoned if it turns blue color. When OD value of standard (concentration: 0)＜0.8unit (A450nm＜0.8), it indicates the reagent may be 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.