AFM1 (Aflatoxin M1) ELISA Kit

Catalog No: E-TO-E018

96T/96T*3

Version Number:	V1.4
Replace version:	V1.3
Revision Date:	2025.05.21

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.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017
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 Competitive-ELISA as the method for the quantitative detection. It can detect Afflation M1 (AFM1) in samples, such as milk, yogurt, etc. This kit is composed of ELISA Microtiter plate, standard and other supplementary reagents. The microtiter plate 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 substrate reagent 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

Reaction mode (Incubation time and temperature)**:** 25℃; 40min, 15min.

Detection limit: Milk---0.01 ppb; Yogurt (method 2), Milk powder, Whey protein powder, Demineralized whey powder, Milk protein powder, Condensed milk---0.1 ppb;Cheese, Single Cream, Yogurt---0.125 ppb.

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 mL each (ppb=ng/mL=ng/g) (0 ppb, 0.005 ppb, 0.015 ppb, 0.045 ppb, 0.135 ppb, 0.405ppb)
HRP Conjugate Diluent	10 mL
100×Concentrated HRP Conjugate	50 µL
Antibody working solution	5 mL
Substrate Reagent	11 mL
Stop Solution	7 mL
10×Concentrated Wash Buffer	50 mL
Sample Diluent	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 materials required but not supplied

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

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

Reagents: Methanol, $Na_2HPO_4 \cdot 12H_2O$.

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. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid th e kit to strong light.
5. **Each reagent is optimized for use in the E-TO-E018. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-TO-E018 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue color. When OD value of standard (concentration: 0) < 0.8 unit (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. **For mentioned sample fast and efficient extraction methods are included in the kit descriptio- n. Please consult technical support for the applicability if other sample need to be tested.**
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 expiry date

Store the kit at 2-8℃. Do not freeze any test kit components.

Return any unused microwells to their original foil bag and reseal them together with the desiccant provided and further store at 2-8℃.

Expiry date: expiration date is on the packing box.

Experimental preparation

Restore 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**

   Please prepare solution according to the number of samples. Don't use up all components at once!

   Solution 1: HRP Conjugate

   Dilute **100×Concentrated HRP Conjugate** with **HRP Conjugate Diluent** (100×Concentrated HRP Conjugate (V): HRP Conjugate Diluent (V) = 1:99), Mix fully (Prepare the fresh solution before use, HRP Conjugate cannot be stored).

   Solution 2: Wash Buffer

   Dilute **10×Concentrated Wash Buffer** with deionized water (10×Concentrated Wash Buffer (V): Deionized water (V) = 1:9).

   Solution 3: 0.2 M Na_2HPO_4 solution

   Accurately weigh 71.63 g of $Na_2HPO_4 \cdot 12H_2O$ and dissolve it in 1 L of deionized water, mix thoroughly.

3. **Sample pretreatment procedure**

3.1 Pretreatment of milk sample:

(1) Take 40 µL of sample for analysis.

Note: Sample dilution factor: 1, detection limit: 0.01 ppb.

3.2 Pretreatment of single cream, cheese, yogurt sample:

(1) Homogenize the fresh sample with a homogenizer.

(2) Weigh 1±0.05 g of homogenate sample into centrifuge tube, add 1 mL of **Methanol**, vortex for 30 s. Centrifuge at 4000 g for 5 min at room temperature.

(3) Take 40 µL of supernatant into another centrifuge tube, and add 460 µL of **Sample Diluent**, vortex for 30 s and mix fully.

(4) Take 40 µL of for analysis.

Note: Sample dilution factor: 25, detection limit: 0.125 ppb.

3.3 Pretreatment of milk powder sample:

(1) Weigh 1±0.05 g of milk powder sample into 15 mL centrifuge tube, add 5 mL of deionized water. Vortex for 2 min, mix fully.

(2) Take 40 µL for analysis.

Note: Sample dilution factor: 5, detection limit: 0.1 ppb.

3.4 Pretreatment of whey protein powder, demineralized whey powder, milk protein powder, condensed milk sample:

(1) Weigh 1±0.05 g of milk powder sample into 15 mL centrifuge tube, add 5 mL of deionized water. Vortex for 2 min, mix fully.

(2) Immediately take 500 μL of sample solution and add into 500 μL of deionized water, then mix thoroughly.

(3) Take 40 μL for analysis.

Note: Sample dilution factor: 12, detection limit: 0.1 ppb.

3.5 Pretreatment of yogurt (method 2) sample:

(1) Weigh 1±0.05 g of yogurt sample into 5 mL centrifuge tube, add 1 mL of 0.2 M Na_2HPO_4 solution, vortex for 2 min, mix fully.

(2) Centrifuge at 4000 g for 5 min.

(3) Take 50 μL supernatant liquid into a new centrifuge tube, then add 450 μL sample diluent, vortex for 30 s, mix fully.

(4) Take 40 μL for analysis.

Note: Sample dilution factor: 20, detection limit: 0.1 ppb.

Assay procedure

Restore all reagents and samples to room temperature (25℃) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8℃.

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 40 μL of **Standard** or **Sample** per well, add 40 μL **Antibody working solution**, and then add 40 μL **HRP Conjugate** (Solution 1) in sequence.
3. **Incubation:** cover the plate with sealer. Oscillate for 10 s gently to mix thoroughly, incubate at 25℃ for 40 min in shading light.
4. **Wash:** uncover the sealer carefully, remove the liquid of each well. Immediately add 350 μL of **Wash Buffer** (Solution 2). Repeat wash procedure for 4 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).
5. **Color Development:** add 100 μL of **Substrate Reagent** to each well. Gently oscillate for 10 s to mix thoroughly. Incubate at 25℃ for 15 min in shading light. (The reaction time can be extended according to the actual color change).
6. **Stop reaction:** add 50 μL of **Stop Solution** to each well. Gently oscillate for 10 s to mix thoroughly.
7. **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 concentration on the x-axis, adopting four-parameter logic function to draw a 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.**

Aflatoxin M1 (E-TO-E018) **Standard Curve**