AF (Total Aflatoxin) ELISA Kit

Catalog No: E-TO-E028

96T/96T*3

Version Number:	V1.5
Replace version:	V1.4
Revision Date:	2024.11.06

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.

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 Total Aflatoxin (AF) in samples, such as grain, feed, edible oil, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody working solution, standard liquid and other supplementary reagents. The microtiter plate in this kit has been pre-coated with coupled antigen. During the reaction, AF in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti-AF antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each microtiter plate well, and substrate reagent is added for color development. There is a negative correlation between the OD value of samples and the concentration of AF. The concentration of AF 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℃, 30 min, 15 min.

Detection limit: Grain ---0.72 ppb; Feed, Chinese herbal medicine, Seasoning, Spice powder ---0.9 ppb; Edible oil---0.3 ppb; Muscle (beef, pork), Ham sausage---0.06 ppb; Milk---0.06 ppb.

Cross-reactivity: Aflatoxin B1 (AFB1) ---100%; AFB2---39%; AFG1---100%; AFG2---13.2%; AFM1---6.6%; AFM2--- < 1.0%

Sample recovery rate: 90±30%

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1 mL each (ppb=ng/mL=ng/g) (0 ppb, 0.03 ppb, 0.09 ppb, 0.27 ppb, 0.81 ppb, 2.43 ppb)
HRP Conjugate	6 mL
Antibody Working Solution	6 mL
Substrate Reagent	11 mL
Stop Solution	6 mL
5×Concentrated Sample Diluent	30 mL
20×Concentrated Wash Buffer	30 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

Instrument: Microtiter plate 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: HCl, $ZnSO_4 \cdot 7H_2O$, Methanol, Ethyl acetate, Dichloromethane, N- hexane, Acetonitrile

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 the kit to strong light.
5. **Each reagent is optimized for use in the E-TO-E028. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-TO-E028 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 ($A_{450\ nm}$ < 0.8), 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. **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 cros s-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: 1 M HCl

Dilute 8.6 mL of **HCl** to 100 mL with deionized water, mix fully.

Solution 2: Sample Solution

Add 200 mL of **Methanol** and 1 mL of **1 M HCl** (Solution 1), mix fully.

Solution 3: 1 M ZnSO4 Solution

Dissolve 16.15 g of **$ZnSO_4$** to 100 mL with deionized water.

Solution 4: Mixed Solution

Add 400 mL of **Ethyl acetate** and 100 mL of **Dichloromethane**, mix fully.

(Ethyl acetate (V): Dichloromethane (V) =4:1).

Solution 5: Sample Diluent A

Dilute the **10×Concentrated Sample Diluent** with deionized water. (10×Concentrated Sample Diluent (V): Deionized water (V) =1:9), mix fully.

Solution 6: Sample Diluent B

Add 30 mL of **Methanol** and 70 mL of **Sample Diluent A** (Solution 5), mix fully.

Solution 7: Sample Diluent C

Dilute the **10×Concentrated Sample Diluent** with deionized water (10×Concentrated Sample Diluent (V): Deionized water (V) =1:4), mix fully.

Solution 8: Sample Diluent D

Dilute the **20×Concentrated Wash Buffer** with deionized water (20×Concentrated Wash Buffer (V): Deionized water (V) =1:39), mix fully.

Solution 9: Wash Buffer

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

3. Sample pretreatment procedure

Substance in sample is distributed unevenly. It is recommended that more samples should be taken when sampling.

3.1 Pretreatment of feed, Chinese herbal medicine sample:

(1) Homogenize the representative sample with a homogenizer and mix fully.

(2) Weigh 1±0.05 g of crushed homogenate into 50 mL centrifuge tube, add 5 mL of **Sample Solution** (Solution 2), vortex for 1 min, centrifuge at 4000 r/min for 5 min at room temperature.

(3) Take 100 μL of supernatant to another centrifuge tube, add 400 μL of **Sample Diluent C** (Solution 7), mix fully.

(4) Take 50 μL for analysis.

Note: Sample dilution factor: 30, Detection limit: 0.9 ppb

3.2 Pretreatment of edible oil sample:

(1) Take 0.1 mL of sample into 4 mL centrifuge tube, add 1 mL of **N-hexane** and 1 mL of **Sample Diluent B** (Solution 6), vortex for 1 min.

(2) Centrifuge at 4000 r/min for 5 min at room temperature, discard the upper and middle liquid.
(3) Take 50 μL of lower layer liquid for analysis.
Note: Sample dilution factor: 10, Detection limit: 0.3 ppb

3.3 Pretreatment of grain sample:
(1) Weigh 1 ±0.05 g of crushed homogenate into 50 mL centrifuge tube, add 5 mL of **Methanol**, oscillate for 1 min, centrifuge at 4000 r/min for 5 min at room temperature.
(2) Take 100 μL of supernatant to another centrifuge tube, Add 300 μL of **Sample Diluent D** (Solution 8). Vortex for 2 min.
(3) Take 50 μL for analysis.
Note: Sample dilution factor: 24, Detection limit: 0.72 ppb

3.4 Pretreatment of seasoning (soy sauce, chilli powder, cumin powder), peanut sample:
(1) Weigh 1 ±0.05 g of crushed homogenate into 50 mL centrifuge tube, add 5 mL of **Methanol**, vortex for 1 min, centrifuge at 4000 r/min for 5 min at room temperature.
(2) Take 100 μL of supernatant to another centrifuge tube.
(3) **Chilli powder:** Add 400 μL of **Sample Diluent A** (Solution 5), vortex for 5 min;
Soy sauce, peanut: Add 400 μL of deionized water, vortex for 5 min;
Cumin powder: Add 400 μL of **Sample Diluent C** (Solution 7), vortex for 5 min.
(4) Take 50 μL for analysis.
Note: Sample dilution factor: 30, Detection limit: 0.9 ppb

3.5 Pretreatment of spice powder (Illicium verum, ground pepper) sample:
(1) Weigh 1 ±0.05 g of crushed homogenate into 50 mL centrifuge tube, add 5 mL of **Acetonitrile**, vortex for 5 min, centrifuge at 4000 r/min for 5 min at room temperature.
(2) Take 100 μL of supernatant to another centrifuge tube.
(3) Add 1 mL of **N- hexane** and 400 μL of **Sample Diluent C** (Solution 7), vortex for 5 min.
(4) Centrifuge at 4000 r/min for 5 min at room temperature, discard the upper and middle liquid.
(5) Take 50 μL of lower layer liquid for analysis.
Note: Sample dilution factor: 30, Detection limit: 0.9 ppb

3.6 Pretreatment of milk sample:
(1) Take 2 mL of liquid milk into 50 mL centrifuge tube, add 0.4 mL of **1 M ZnSO4 Solution** (Solution 3), 6 mL of **Mixed Solution** (Solution 4) vortex for 5 min, centrifuge at 4000 r/min for 5 min at room temperature.
(2) Take 1.5 mL of supernatant to another centrifuge tube, dry with nitrogen evaporators or water bath at 50℃ (Please do it in a ventilated environment).
(3) Dissolve the residual with 2 mL of **N- hexane**, and add 1 mL of **Sample Diluent B** (Solution 6).Vortex for 1 min.
(4) Centrifuge at 4000 r/min for 5 min at room temperature, discard the upper and middle liquid.

(5) Take 50 μL of lower layer liquid for analysis.
Note: Sample dilution factor: 2, Detection limit: 0.06 ppb

3.7 Pretreatment of muscle (beef, pork), ham sausage sample:

(1) Weigh 2±0.05 g of crushed homogenate into 50 mL centrifuge tube, add 8 mL of **Ethyl acetate**, vortex for 30 min, and centrifuge at 4000 r/min for 10 min at room temperature.
(2) Take 2 mL of supernatant to another centrifuge tube, dry with nitrogen evaporators or water bath at 50℃ (Please do it in a ventilated environment).
(3) Dissolve the residual with 2 mL of **N- hexane**, vortex for 1 min, add 1 mL of **Sample Diluent A** (Solution 5).
(4) Centrifuge at 4000 r/min for 5 min at room temperature, discard the upper and middle liquid. Standing for 15min.
(5) Take 50 μL of lower layer liquid for analysis.
Note: Sample dilution factor: 2, Detection limit: 0.06 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. 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 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 with sealer, oscillate for 10 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 260 μL of **Wash Buffer** (Solution 9) to each well and wash. 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).
4. **Color Development:** add 50 μL of **Substrate Reagent A** to each well, and then add 50 μL of **Substrate Reagent B**. Gently oscillate for 10 s to mix thoroughly. Incubate shading light for 15 min at 25℃ (The reaction time may be shortened or prolonged according to the depth of the color).
5. **Stop reaction:** add 50 μL of **Stop Solution** to each well, oscillate for 10s 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 5 min after stop reaction.

Result analysis

1. **Drawing and calculation of standard curve**
Create a standard curve by plotting the absorbance of each standard on the y-axis against the concentration on the x-axis to draw a four-parameter fit 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 form for accurate and fast analysis on a large number of samples.

Total Aflatoxin (E-TO-E028) **Standard Curve**