AF (Total Aflatoxin) ELISA Kit

Catalog No: E-TO-E006

96T/96T*3

Version Number:	V1.2
Replace version:	V1.1
Revision Date:	2024.03.14

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
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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 Total Aflatoxin (AF) in samples, such as cereals, formula feed, edible oil, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody working solution, standard 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: Cereals---0.1 ppb; Formula feed ---0.2 ppb; Edible oil, Peanut---0.2 ppb; Biscuit ---0.2 ppb; Beer---0.2 ppb; Wine, Soy sauce, Vinegar---0.1 ppb

Cross-reactivity: Aflatoxin B1 (AFB1) ---100%, Aflatoxin B2 (AFB2) ---80%, Aflatoxin G1 (AFG1) ---75%, Aflatoxin G2 (AFG2) ---45%, Aflatoxin M1 (AFM1) ---8%

Sample recovery rate: Cereals, Formula feed---85%±15%; Peanut---82%±15%; Edible oil---85%±15%; Biscuit---83%±15%; Beer---84%±15%; Wine, Soy sauce, Vinegar---87%±15%.

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1 mL each (ppb=ng/mL=ng/g) (0 ppb, 0.02 ppb, 0.04 ppb, 0.08 ppb, 0.16 ppb, 0.32 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
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: Microplate reader, Printer, Homogenizer, Nitrogen Evaporators, Water bath, Vortex mixer, Centrifuge, Graduated pipette, Balance (sensibility 0.01 g).

High-precision transferpettor: Single channel (20-200 μL, 100-1000 μL), Multichannel (300 μL).

Reagents: Methanol, N-hexane, Trichloromethane or Dichloromethane.

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-E006. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-TO-E006 with different lot numbers**.
6. Substrate Reagent should be abandoned if it turns blue color. When OD value of standard (concentration: 0) < 0.5 unit (A_{450nm} < 0.5), 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 use disposable pipette tips 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 in the kit at once!

   Solution 1: 70% Methanol

   Methanol (V): Deionized water (V) =7:3.

   Solution 2: Wash Buffer

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

3. **Sample pretreatment**

3.1 Pretreatment of cereals sample:

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

(2) Weigh 2±0.05 g of homogenate sample into the 50 mL centrifuge tube, add 5 mL of **70% Methanol** (Solution 1), vortex for 5 min, centrifuge at 4000 r/min for 10 min at room temperature.

(3) Take 0.5 mL of supernatant to another centrifuge tube, add 0.5 mL of deionized water, and mix fully.

(4) Take 50 μL for analysis.

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

3.2 Pretreatment of formula feed sample:

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

(2) Weigh 2±0.05 g of homogenate sample into the 50 mL centrifuge tube, add 10 mL of **70% Methanol** (Solution 1), vortex for 5 min, centrifuge at 4000 r/min for 10 min at room temperature.

(3) Take 0.5 mL of supernatant to another centrifuge tube, add 0.5 mL of deionized water, and mix fully.

(4) Take 50 μL for analysis.

Note: Sample dilution factor: 10, detection limit: 0.2 ppb

(If aflatoxin content is higher in the sample, take the mixed liquid from step 2, diluted with **35% Methanol**, the sample dilution multiple is the actual dilution multiple at the moment. For example: take the mixed liquid from step 2, diluted 10 times with **35% Methanol**, the actual dilution multiple is 10×10=100, detection limit: 2 ppb)

3.3 Pretreatment of edible oil, peanut, high fat formula feed sample:

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

(2) Weigh 2±0.05 g of homogenate sample into the 50 mL centrifuge tube, add 8 mL of **N-hexane** and 10 mL of **70% Methanol** (Solution 1), vortex for 5 min, centrifuge at 4000 r/min for 10min at room temperature.

(3) Discard the upper liquid, and take 0.5 mL of lower liquid to another centrifuge tube, add 0.5 mL of deionized water, mix fully.

(4) Take 50 μL for analysis.

Note: Sample dilution factor: 10, detection limit: 0.2 ppb

3.4 Pretreatment of biscuit sample:

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

(2) Weigh 2±0.05 g of homogenate sample into the 50 mL centrifuge tube, add 10 mL of **70% Methanol** (Solution 1), vortex for 5 min, centrifuge at 4000 r/min for 10 min at room temperature.

(3) Take 2 mL of supernatant to another centrifuge tube, add 4 mL of **Trichloromethane** or **Dichloromethane**, vortex for 5 min, centrifuge at 4000 r/min for 10 min at room temperature.

(4) Take the upper liquid to another centrifuge tube, keep the lower liquid for use (lower liquid A). Add 4 mL of **Trichloromethane or Dichloromethane** to the upper liquid, vortex sufficiently for 5 min, and centrifuge at 4000 r/min for 10 min at room temperature. Discard the upper liquid and keep the lower liquid (lower liquid B).

(5) Mix lower liquid A and lower liquid B thoroughly.

(6) Take 2 mL of mixed lower liquid and dry with nitrogen evaporators or water bath at 50-60℃(Please do it in a ventilated environment.).

(7) Add 0.5 mL of **70% Methanol** (Solution 1) to dissolve thoroughly, add 0.5 mL of deionized water, and mix fully.

(8) Take 50 μL for analysis.

Note: Sample dilution factor: 10, detection limit: 0.2 ppb

3.5 Pretreatment of beer sample:

(1) Stir beer thoroughly to remove CO_2, take 2 mL of beer sample and add 1 mL of deionized water, then add 7 mL of **Methanol**, vortex for 5 min.

(2) Take 0.5 mL of mixed sample liquid and add 0.5 mL of deionized water to another centrifuge tube, mix fully.

(3) Take 50 μL for analysis.

Note: Sample dilution factor: 10, detection limit: 0.2 ppb

3.6 Pretreatment of wine, soy sauce, vinegar sample:

(1) Take 2 mL of sample and add 2 mL of deionized water, then add 10 mL of **Trichloromethane or Dichloromethane**, vortex for 5 min, centrifuge at 4000 r/min for 10 min at room temperature.

(2) Remove all upper liquid. Take 1 mL of lower liquid to another centrifuge tube and dry with nitrogen evaporators or water bath at 50-60℃.(Please do it in a ventilated environment.)

(3) Add 0.5 mL of **70% Methanol** (Solution 1) to dissolve thoroughly, add 0.5 mL of deionized water, and mix fully.

(4) Take 50 μL for analysis.

Note: Sample dilution factor: 5, 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 together to sealed bag with the desiccant provided 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 plate sealer, oscillate for 5 s gently to mix thoroughly, incubate for 30 min at 25℃ in shading light.
3. **Wash:** uncover the sealer carefully, remove the liquid. Immediately add 300 μL of **Wash Buffer** (Solution 2) 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 Reagent A** to each well, and then add 50 μL of **Substrate Reagent B**. Gently oscillate for 5 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).
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 samples

   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 form for accurate and fast analysis on a large number of samples.