Elabscience®

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AF (Total Aflatoxin) ELISA Kit

Catalog No: E-TO-E006

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.

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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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Test principle

This kit uses Competitive-ELISA as the method. It can detect Aflatoxin (AF) in samples, such as grain, peanut, formula feed, etc. 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, 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 micro plate well, and TMB substrate 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

Sensitivity: 0.02 ppb (ng/mL)

Reaction mode: 25℃, 30 min~ 15 min

Detection limit: Grain---0.1 ppb; Formula feed---0.2 ppb; Edible oil/Peanut---0.2 ppb;
Sauce/Wheat/Barley feed---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: Grain/Formula feed---85% ± 15%, Peanut---82% ± 15%,
Edible oil---85% ± 15%, Sauce/Wheat/Barley feed---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 (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 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: Methanol, N-hexane, Chloroform or Dichloromethane.

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 use disposable pipette tips to avoid cross-contamination during the experiment.

2. Solution preparation

Solution 1: 70% Methanol.

Methanol (Volume): Deionized water (Volume) =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 grain:

(1) Weigh 2 g of crushed homogenate into the 50 mL EP tube, add 5 mL of **70% methanol**, oscillate for 5 min, centrifuge at 4000 rpm for 10 min at room temperature;

(2) Take 0.5 mL of supernatant, add 0.5 mL of deionized water, mix fully;

(3) Take 50 μL for detection and analysis.

Note: Sample dilution factor: 5, minimum detection dose: 0.1 ppb

3.2. Pretreatment of formula feed:

(1) Weigh 2 g of crushed homogenate into the 50 mL EP tube, add 10 mL 70% of methanol, oscillate for 5 min, centrifuge at 4000 rpm for 10 min at room temperature;

(2) Take 0.5 mL of supernatant, add 0.5 mL of deionized water, mix fully;

(3) Take 50 μL for detection and analysis.

Note: Sample dilution factor: 10, minimum detection dose: 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% of methanol, the actual dilution multiple is 10×10=100, detection limit: 2 ppb)

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

(1) Weigh 2 g of crushed homogenate into the 50 mL EP tube, add 8 mL of N-hexane and 10 mL 70% of methanol, oscillate for 5 min, centrifuge at 4000 rpm for 10min at room temperature;

(2) Discard the upper liquid, and take 0.5 mL of lower liquid, add 0.5 mL of deionized water, mix fully;

(3) Take 50 μL for detection and analysis.

Note: Sample dilution factor: 10, minimum detection dose: 0.2 ppb

3.4. Pretreatment of Sauce/Wheat/Barley feed：

(1) Weigh 2 g of crushed homogenate into the 50 mL EP tube, add 10 mL of 70% methanol, oscillate for 5 min, centrifuge at 4000 rpm for 10 min at room temperature;

(2) Take 2 mL of supernatant, add 4 mL of trichloromethane or dichloromethane, oscillate for 5 min, centrifuge at 4000 rpm for 10 min at room temperature;

(3) Take the upper liquid to another vessel, keep the lower liquid for use (lower liquid A). Add 4 mL of trichloromethane or dichloromethane to the upper liquid, oscillate sufficiently for 5 min, centrifuge at 4000 rpm for 10 min at room temperature. Discard the upper liquid and keep the lower liquid (lower liquid B);

(4) Mix lower liquid A and lower liquid B thoroughly;

(5) Take 2 mL of mixed lower liquid and blow-dry with nitrogen evaporators/water bath at 50-60℃;

(6) Add 0.5 mL of 70% methanol to dissolve thoroughly, add 0.5 mL of deionized water, mix fully;

(7) Take 50 μL for detection and analysis.

Note: Sample dilution factor: 10, minimum detection dose: 0.2 ppb

3.5. Pretreatment of beer:

(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 methanol, oscillate for 5 min;

(2) Take 0.5 mL of mixed sample liquid and add 0.5 mL of deionized water, mix fully;

(3) Take 50 μL for detection and analysis.

Note: Sample dilution factor: 10, minimum detection dose: 0.2 ppb

3.6. Pretreatment of wine, soy sauce, vinegar:

(1) Take 2 mL of sample and add 2 mL of deionized water, then add 10 mL of trichloromethane or dichloromethane, oscillate for 5 min, centrifuge at 4000 rpm for 10 min at room temperature;

(2) Take 1 mL of lower liquid and blow-dry with nitrogen evaporators/water bath at 50-60℃;

(3) Add 0.5 mL of 70% methanol to dissolve thoroughly, add 0.5 mL of deionized water, mix fully;

(4) Take 50 μL for detection and analysis.

Note: Sample dilution factor: 5, minimum detection dose: 0.1 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 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 sealer, oscillate for 5 s gently to mix thoroughly, incubate for 30 min at 25℃.
3. **Wash:** uncover the sealer carefully, remove the liquid. 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 reagent A** to each well, and then add 50 μL of **substrate reagent B**. Gently oscillate for 5 s to mix thoroughly. Incubate 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 with a microplate reader (the 450/630 nm double wavelength is recommended). This step should be finished in 10 min after stop reaction.

Result analysis

1. **Absorbance (%) =$A/A_0 \times 100\%$**

   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 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)＜0.5 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. 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

Storage: Store at 2-8℃ for 1 year.. Avoid freezing.

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

Valid Period: expiration date is on the packing box.

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