Elabscience®

7th Edition, revised inApril, 2017

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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.

Phone: 240-252-7368(USA)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 Indirect-Competitive-ELISA as the method. It can detect Aflatoxin(AF) in samples, such as grain, peanut, feed, etc. This kit is composed of Micro ELISAPlate, HRP conjugate, antibody, standard and other supplementary reagents. The micro-plate provided in this kit has been pre-coated with AF. During the reaction, AF in the samples or standard competes with AFon the solid phase supporter for sites of AF antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well, and TMB substrate is 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:0.02ppb (ng/mL)

Reaction mode: 25℃, 30min~ 15min

Detection limit: Grain---0.1ppb; Formula feed---0.2ppb; Edibleoil/Peanut---0.2ppb;
Sauce/Wheat/Barley feed---0.2ppb; beer---0.2ppb; Wine/Soy sauce/Vinegar---0.1ppb

Cross-reactivity: Aflatoxin B1 (AFB1) ---100%,AflatoxinB2 (AFB2) ---80%,
AflatoxinG1 (AFG1)--75%, AflatoxinG2 (AFG2) ---45%, FlatoxinM1 (AFM1) ---8%

Sample recovery rate: Grain/ Formula feed---85%±15%, Peanut---82%±15%, Edibleoil---85%±15%
Sauce/Wheat/Barleyfeed---83%±15%, Beer---84%±15%,
Wine/Soy sauce/Vinegar---87%±15%

Kits components

Item	Specifications
Micro ELISA Plate	96 wells
Standard Liquid(black cap)	1mL each (0ppb,0.02ppb,0.04ppb,0.08ppb,0.16ppb,0.32ppb)
High ConcentratedStandard (100ppb)	1mL
HRP Conjugate(red cap)	5.5mL
Antibody Working Solution(blue cap)	5.5mL
Substrate Reagent A(white cap)	6mL
Substrate Reagent B(black cap)	6mL
Stop Solution(yellow cap)	6mL
20×Concentrated Wash Buffer(white cap)	40mL
Product Description	1 copy

Other supplies required

Instrument: Micro-plate reader, Printer, Homogenizer, Nitrogen Evaporators, Oscillators, Centrifuge, Graduated pipette, Balance (sensibility 0.01g).

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

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

Experimental preparation

Bring all reagents and samples to room temperature before use.

Open the micro-plate 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) =70:30

3. **Sample pretreatment**

3.1. Pretreatment of grain:

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

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

(3) Take 50μL for detection and analysis.

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

3.2. Pretreatment of formula feed:

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

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

(3) Take 50μLfor detection and analysis.

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

(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: 2ppb)

3.3. Pretreatmentofedible oil, peanut, high fat feed:

(1) Weigh 2g of crushedhomogenate into the 50mL EP tube, add 8mL of N-hexane and 10mL70% of methanol, oscillate for 5min, centrifuge at 4000r/min for 10min at room temperature;

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

(3) Take 50μLfor detection and analysis.

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

3.4. Pretreatmentof sauce, wheat and barley, biscuit, pastry, flavour and feed concentrates:

(1) Weigh 2g of crushedhomogenate into the 50mL EP tube, add 10mL of 70% methanol, oscillate for 5min, centrifuge at 4000r/min for 10min at room temperature;
(2) Take 2mL of supernatant, add 4mL of chloroform or dichloromethane, oscillate for 5min, centrifuge at 4000r/min for 10min at room temperature;
(3) Take the upper liquid to another vessel, keep the lower liquid for use (lower liquid A). Add 4mL of chloroform or dichloromethane to the upper liquid, oscillate sufficiently for 5min, centrifuge at 4000r/min for 10min 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 2mL of mixed lower liquid and blow-dry with Nitrogen Evaporators at 50-60℃;
(6) Add 0.5mL of 70% methanol to dried materials to dissolve thoroughly, add 0.5mL of deionized water, mix;
(7) Take 50μL for detection and analysis.

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

3.5. Pretreatment of beer:

(1) Stir beer thoroughly to remove CO_2, take 2mL of beer sample and add 1mL of deionized water, then add 7mL of 70% methanol, oscillate for 5min;
(2) Take 0.5mL of mixed sample liquid and add 0.5mL of deionized water, mix;
(3) Take 50μL for detection and analysis.

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

3.6. Pretreatment of wine, soy sauce, vinegar:

(1) Take 2mL of sample and add 2mL of deionized water, then add 10mL of chloroform or dichloromethane, oscillate for 5min, centrifuge at 4000r/min for 10min at room temperature;
(2) Take 1mL of lower liquid and blow-dry with Nitrogen Evaporators at 50-60℃;
(3) Add 0.5mL of 70% methanol to dried materials to dissolve thoroughly, add 0.5mL of deionized water, mix;
(4) Take 50μL for detection and analysis.

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

Assay procedure

Centrifuge the sample again after thawing before the assay. Bring all reagents to room temperature for 30 min before use.**All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.**Dilute40mL of concentrated wash buffer into 800mLwash working buffer with deionized or distilled water.

1. **Number:** number the sample and standard in order (multiplewell), and keep a record of standard wells and sample wells.
2. **Add sample:** add 50μL of Standard, Blank, or Sample per well, then add 50μL of HRP conjugate to each well, then add 50μL of antibody working solution, cover the plate with sealer we provided, oscillate for 5s gently to mix thoroughly, incubate for 30min at 25℃.
3. **Wash:** uncover the sealer carefully,remove the liquid in each well. Immediately add 250μL of wash working buffer to each well and wash. Repeat wash procedure for 5 times, 30s 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 50μL of substrate solution A to each well, and then add 50μL of substrate solution B. Gently oscillate for 5s to mix thoroughly. Incubate shading light for 15min at 25℃.
5. **Stop reaction:** add 50μL 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 micro-plate reader (the 450/630 nm double wavelength is recommended). This step should be finished in 10min after stop reaction.

Result analysis

1. **Absorbance (%)=A/A_0×100%**

   A: Average absorbance of standard or sample

   A_0: Average absorbance of 0ppb 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 valueof sampleto 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. Overall OD value will be lower when reagents is not brought to room temperature before use or room temperature is below25℃.
2. During the washing procedure, if the wells turn dry, it will lead to bad linear standard curve and poor repeatability, move on to 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. Micro ELISA plate should be covered by plate sealer. Avoid the reagents to strong light.
5. Do not use expired kit and reagents of different batches.
6. TMB should be abandoned if it turns color. When OD value of standard(concentration: 0)＜0.5 unit(A_{450nm}＜0.5), it indicates reagent is deteriorated.
7. Stop solution is caustic, avoidcontact withskinandeyes.

Storage and valid period:

Storage: Store at 2-8℃. Avoid freeze / thaw cycles.

Valid Period: 1 year, production date is on the packing box.