Elabscience®

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Aflatoxin B1（AFB1）ELISA Kit

Catalog No: E-TO-E010

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)
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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 Indirect-Competitive-ELISA as the method. It can detect Aflatoxin B1(AFB1) in samples, such as grain and feed,etc. This kit is composed of Micro ELISA Plate, HRP conjugate, antibody, standard and other supplementary reagents. The Microplate provided in this kit has been pre-coated with AFB1. During the reaction, AFB1 in the samples or standard competes with AFB1 on the solid phase supporter for sites of AFB1 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 AFB1. The concentration of AFB1 in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Sensitivity: 0.01ppb (ng/mL)
Reaction mode: 25℃, 30min～15min
Detection limit: Grain and processed products---0.03 ppb
Cross-reactivity: Aflatoxin B1(AFB1) ---100%
Sample recovery rate: Grain and compound feed --85%±15%

Kits components

Item	Specifications
Micro ELISA Plate	96 wells
Standard Liquid(black cap)	1 mL each (0 ppb, 0.01 ppb, 0.02 ppb,0.04 ppb,0.08 ppb, 0.16ppb)
High Standard Solution 100ppb	1 mL
HRPConjugate(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

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

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 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:Sample Extraction Buffer

35% methanol(methanol: deionized water=7:13)

Solution 2: 1×Working Wash Buffer

Dilute the concentrated wash buffer to 20 times (wash buffer: deionizedwater=1:19)

3. Sample pretreatment procedure

Pretreatment ofGrain and processed products:

(1) Weigh 2 g of crushed homogenate into 50mL EP tube, add 6 mL of sample extraction buffer, oscillate for 5min, centrifuge at 4000 r/min for 10 min at room temperature;

(2) Take 50μL of supernatant for analysis.

Note: Sample dilution factor: 3, minimum detection dose: 0.03ppb

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.** Dilute 40 mL of concentrated wash buffer into 800 mL of wash buffer with deionized or distilled water.

1. **Number:** number the sample and standard in order (multiple wells), and keep a record of standard wells and sample wells.
2. **Add sample:** add 50 μL of Standard or Sample per well, then add 50 μL of antibody working solution, cover the plate with sealer we provided, oscillate for 5s gently to mix thoroughly, incubate for 30 min at 37℃.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 250 μL 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. **Add HRP Conjugate:** add 100 μL of HRP conjugate to each well, incubate for 30 min at 37℃.

5. **Wash:** repeat step 3.
6. **Color Development:** add 50 μL of substrate solution A to each well, and then add 50 μL of substrate solution B. Gently oscillate for 5s to mix thoroughly. Incubate shading light for 15min at 37℃. (If the blue color is too shallow, can extend the incubation time properly.
7. **Stop reaction:** add 50 μL of stop solution to each well, oscillate gently to mix thoroughly.
8. **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 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. Overall OD value will be lower when reagents is not brought to room temperature before use or room temperatureis below 25℃.
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($A_{450\ nm}$＜0.5), it indicates reagent is deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.

Storage and valid period

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

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