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**(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !)**

## **AFB1 (Aflatoxin B1) ELISA Kit**

Catalog No: E-TO-E008

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](mailto:techsupport@elabscience.com)

Website: [www.elabscience.com](http://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 AflatoxinB1 (AFB1) in samples, such as grain, peanut, feed, etc. This kit is composed of Micro ELISA Plate, HRP conjugate, antibody, standard and other supplementary reagents. The micro-plate 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:** 0.03ppb (ng/mL)

**Reaction mode:** 25°C, 30min~ 15min

**Detection limit:** Grain---0.15ppb, Formula feed---0.3ppb, Edible oil/Peanut---0.6ppb,  
Sauce/Wheat/Barley feed---0.3ppb, Beer---0.3ppb,  
Wine/Soysauce/Vinegar---0.15ppb

**Cross-reactivity:** AflatoxinB1 (AFB1) 100%

**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
Micro ELISA Plate	96 wells
Standard Liquid (black cap)	1mL each (0ppb, 0.03ppb, 0.06ppb, 0.12ppb, 0.24ppb, 0.48ppb)
High Concentrated Standard (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 (sensitivity 0.01g).

**High-precision transferpette:** single channel (20-200 $\mu$ L, 100-1000 $\mu$ L), Multichannel (300 $\mu$ L).

**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 the pipette should be disposable to avoid cross-contamination during the experiment.

### 2. Solution preparation

Solution 1 (sample extraction solution): 70% methanol. Methanol (volume): Deionized water (volume) = 7:3

Solution 2 (1 $\times$  wash working buffer): 20 $\times$  Concentrated Wash Buffer (volume): Deionized water (volume) = 1:19.

### 3. Sample pretreatment procedure

#### 3.1 Pretreatment of grain:

- (1) Weigh 2g of crushed homogenate into 50mL EP tube, add 5mL of 70% 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 50 $\mu$ L for detection and analysis.

**Note: Sample dilution factor: 5, minimum detection dose: 0.15ppb**

#### 3.2 Pretreatment of corn husk, wheat bran and other strong water absorption samples:

- (1) Weigh 2g of crushed homogenate into 50mL EP tube, add 20mL of 70% methanol, oscillate for 5min, centrifuge at 4000r/min for 10min at room temperature;
- (2) Take 0.5mL of supernatant, mix with 0.5mL of deionized water.

For the sample containing high level of toxins, it can be diluted by 35% methanol before determination. For example, take 0.1mL of the mixed solution in the procedure 3.2 (2), add 0.9mL of 35% methanol, mix up. The final dilution factor of sample is 200, the minimum detection dose is 6ppb.

- (3) Take 50 $\mu$ L for detection and analysis.

**Note: Sample dilution factor: 20, minimum detection dose: 0.6ppb**

As the toxins distribute unevenly in samples, we need make multidraw and mix fully first, then take 2 g of the solution to detect.

### **3.3 Pretreatment of formula feed:**

- (1) Weigh 2g of crushed homogenate into 50mL EP tube, add 10mL of 70% 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 50μL for detection and analysis.

**Note: Sample dilution factor: 10, minimum detection dose: 0.3ppb**

### **3.4 Pretreatment of edible oil, peanut, high fat feed:**

- (1) Weigh 2g of crushed homogenate into 50mL EP tube, add 8mL of N-hexane and 10mL of 70% methanol, oscillate for 5min, centrifuge at 4000r/min for 10min at room temperature;
- (2) Discard the upper liquid, and take 0.5mL of lower liquid, add 0.5mL of deionized water, mix;
- (3) Take 0.5mL of mixed liquid from step 2, then add 0.5mL of 35% methanol, oscillate for 30s;
- (4) Take 50μL for detection and analysis.

**Note: Sample dilution factor: 20, minimum detection dose: 0.6ppb**

### **3.5 Pretreatment of sauce, wheat and barley, biscuit, pastry, flavor and feed concentrates:**

- (1) Weigh 2g of crushed homogenate into 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 dry with nitrogen evaporators at 50-60°C;
- (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: 0.3ppb**

### **3.6 Pretreatment of beer:**

- (1) Stir beer thoroughly to remove CO<sub>2</sub>, 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: 0.3ppb**

### **3.7 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 dry with nitrogen evaporators at 50-60°C.
- (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: 0.15ppb**

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

1. **Number:** number the sample and standard in order (multiple well), 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°C.
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°C.
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 (%) = $A/A_0 \times 100\%$

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 temperature is below 25°C.
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 ( $A_{450\text{nm}} < 0.5$ ), 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°C. Avoid freeze / thaw cycles.

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