AFB1 (Aflatoxin B1) ELISA Kit

Catalog No: E-TO-E017

96T/96T*3

Version Number:	V1.2
Replace version:	V1.1
Revision Date:	2026.04.15

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
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 Competitive-ELISA as the method for the quantitative detection. It can detect Aflatoxin B1 (AFB1) in samples, such as grain, feed, edible oil, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody working solution, standard liquid and other supplementary reagents. The microtiter plate in this kit has been pre-coated with coupled antigen. During the reaction, AFB1 in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti-AFB1 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 AFB1. The concentration of AFB1 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, 15-20 min.

Detection limit: Finished feed (swine, bovine), meals (soybean meal, rapeseed meal), cereals (corn flour, corn bran, wheat bran, soybean flour, mung bean flour, red bean flour, rice flour, barley flour, sorghum flour, wheat flour, plain flour, peanut), seasonings (chili powder, cumin powder, soy sauce, fermented black soybean, star anise powder, sichuan pepper powder, black pepper powder), biscuits---3 ppb; Edible oil---1 ppb; Beef, pork, ham sausage---0.5 ppb; Modified milk (grain milk, wheat-flavored breakfast milk)---0.1 ppb.

Cross-reactivity: Aflatoxin B1 (AFB1) ---100%; AFB2---39%; AFG1---100%; AFG2---13.2%; AFM1---6.6%; AFM2--- < 1.0%.

Sample recovery rate: 90±30%.

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1.5 mL each (ppb=ng/mL=ng/g) (0 ppb, 0.03 ppb, 0.09 ppb, 0.27 ppb, 0.81 ppb, 2.43 ppb)
HRP Conjugate	7 mL
Antibody Working Solution	7 mL
Substrate Reagent A	7 mL
Substrate Reagent B	7 mL
Stop Solution	7 mL
10×Concentrated Sample Diluent	40 mL
20×Concentrated Wash Buffer	25 mL
Plate Sealer	1 piece
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: Microtiter plate 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: HCl, $ZnSO_4 \cdot 7H_2O$, Methanol, Ethyl acetate, Dichloromethane, N- hexane, Acetonitrile.

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 the kit to strong light.
5. **Each reagent is optimized for use in the E-TO-E017. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-TO-E017 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue color. When OD value of standard (concentration: 0) < 0.8 unit ($A_{450\ nm} < 0.8$), it indicates reagent is 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 description. 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℃. After opening, the kit is stable for up to 1 month.

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 the pipette should be disposable to avoid cros s-contamination during the experiment.

2. **Solution preparation**

   Please prepare solution according to the number of samples. Don't use up all components at once!

   Solution 1: **1 M HCl**

   Dilute 8.6 mL of **HCl** to 100 mL with deionized water, mix fully.

   Solution 2: **Sample Extraction Solution**

   Add 200 mL of **Methanol** and 1 mL of **1 M HCl** (Solution 1), mix fully.

   Solution 3: **1 M ZnSO4 Solution**

   Dissolve 28.8 g of **$ZnSO_4 \cdot 7H_2O$** to 100 mL with deionized water.

   Solution 4: **Mixed Solution**

   Add 400 mL of **Ethyl acetate** and 100 mL of **Dichloromethane**, mix fully.

   (Ethyl acetate (V): Dichloromethane (V) =4:1).

   Solution 5: **Sample Diluent A**

   Dilute the **10×Concentrated Sample Diluent** with deionized water. (10×Concentrated Sample Diluent (V): Deionized water (V) =1:9), mix fully.

   Solution 6: **Sample Diluent B**

   Add 30 mL of **Methanol** and 70 mL of **Sample Diluent A** (Solution 5), mix fully.

   Solution 7: **Sample Diluent C**

   Dilute the **10×Concentrated Sample Diluent** with deionized water (10×Concentrated Sample Diluent (V): Deionized water (V) =1:4), mix fully.

   Solution 8: **Sample Diluent D**

   Dilute the **20×Concentrated Wash Buffer** with deionized water (20×Concentrated Wash Buffer (V): Deionized water (V) =1:39), mix fully.

   Solution 9: **Wash Buffer**

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

3. Sample pretreatment procedure

Substance in sample is distributed unevenly. It is recommended that more samples should be taken when sampling.

3.1 Pretreatment of finished feed (swine, bovine), meals (soybean meal, rapeseed meal), corn flour, corn bran, wheat bran sample:

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

(2) Weigh 1±0.05 g of crushed homogenate into 50 mL centrifuge tube, add 5 mL of **Sample Solution** (Solution 2), vortex for 1 min, centrifuge at 4000 r/min for 5 min at room temperature.

(3) Take 100 μL of supernatant to another centrifuge tube.

(4) **Finished feed (swine, bovine), corn flour, corn bran, wheat bran:** add 400 μL of **Sample Diluent C** (Solution 7), mix fully.

Meals (soybean meal, rapeseed meal): add 400 μL of **deionized water**, mix fully.

(5) Take 50 μL for analysis.

Note: Sample dilution factor: 30, Detection limit: 3 ppb

3.2 Pretreatment of edible oil sample:

(1) Take 0.1 mL of sample into 4 mL centrifuge tube, add 1 mL of **N-hexane** and 1 mL of **Sample Diluent B** (Solution 6), vortex for 1 min.

(2) Centrifuge at 4000 r/min for 5 min at room temperature, discard the upper and middle liquid.

(3) Take 50 μL of lower layer liquid for analysis.

Note: Sample dilution factor: 10, Detection limit: 1 ppb

3.3 Pretreatment of biscuits sample:

(1) Weigh 1±0.05 g of crushed homogenate into 50 mL centrifuge tube, add 5 mL of **Methanol**, oscillate for 1 min, centrifuge at 4000 r/min for 5 min at room temperature.

(2) Take 100 μL of supernatant to another centrifuge tube, Add 400 μL of **deionized water**,vortex for 1 min.

(3) Take 50 μL for analysis.

Note: Sample dilution factor: 30, Detection limit: 3 ppb

3.4 Pretreatment of cereals (soybean flour, mung bean flour, red bean flour, rice flour, barley flour, sorghum flour, wheat flour, plain flour) sample:

(1) Weigh 1±0.05 g of crushed homogenate into 50 mL centrifuge tube, add 5 mL of **Methanol**, vortex for 1 min, centrifuge at 4000 r/min for 5 min at room temperature.

(2) Take 100 μL of supernatant to another centrifuge tube.

(3) **Soybean flour, mung bean flour, red bean flour, sorghum flour, wheat flour, plain flour:** add 300 μL of **deionized water**, vortex for 1 min.

Rice flour, barley flour: add 300 μL of **Sample Diluent D** (Solution 8), vortex for 1 min.

(4) Take 50 μL for analysis.

Note: Sample dilution factor: 24, Detection limit: 3 ppb

3.5 Pretreatment of peanut, seasonings (chili powder, cumin powder, soy sauce, fermented black soybean) sample:

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

(2) Take 100 µL of supernatant to another centrifuge tube.

(3) **Chili powder, fermented black soybean:** add 400 µL of **Sample Diluent A** (Solution 5), vortex for 1 min.

Peanut, soy sauce: add 400 µL of **deionized water**, vortex for 1 min.

Cumin powder: add 400 µL of **Sample Diluent C** (Solution 7), vortex for 1 min.

(4) Take 50 µL for analysis.

Note: Sample dilution factor: 30, Detection limit: 3 ppb

3.6 Pretreatment of star anise powder, sichuan pepper powder, black pepper powder sample:

(1) Weigh 1±0.05 g of crushed homogenate into 50 mL centrifuge tube, add 5 mL of **Acetonitrile**, vortex for 2 min, and centrifuge at 4000 r/min for 5 min at room temperature.

(2) Take 100 µL of supernatant to another centrifuge tube.

(3) **Star anise powder, black pepper powder:** dissolve the residual with 1 mL of **N- hexane**, vortex for 1 min, add 400 µL of **Sample Diluent C** (Solution 7), vortex for 1 min.

Sichuan pepper powder: dissolve the residual with 1 mL of **N- hexane**, vortex for 1 min, add 900 µL of **Sample Diluent C** (Solution 7), vortex for 1 min.

(4) Centrifuge at 4000 r/min for 5 min at room temperature, discard the upper and middle liquid. Standing for 15min.

(5) Take 50 µL of lower layer liquid for analysis.

Note: Sample dilution factor: Star anise powder, black pepper powder: 30 ; Sichuan pepper powder: 60

Detection limit: Star anise powder, black pepper powder, Sichuan pepper powder: 3 ppb

3.7 Pretreatment of modified milk (grain milk, wheat-flavored breakfast milk) sample:

(1) Take 2 mL of liquid milk into 50 mL centrifuge tube, add 0.4 mL of **1 M ZnSO4 Solution** (Solution 3), 6 mL of **Mixed Solution** (Solution 4) vortex for 1 min, centrifuge at 4000 r/min for 5 min at room temperature.

(2) Take 1.5 mL of supernatant to another centrifuge tube, dry with nitrogen evaporators or water bath at 50-60℃ (Please do it in a ventilated environment).

(3) Dissolve the residual with 2 mL of **N- hexane**, and add 1 mL of **Sample Diluent B** (Solution 6).Vortex for 1 min.

(4) Centrifuge at 4000 r/min for 5 min at room temperature, discard the upper and middle liquid.

(5) Take 50 µL of lower layer liquid for analysis.

Note: Sample dilution factor: 2, Detection limit: 0.1 ppb

3.8 Pretreatment of beef, pork, ham sausage sample:

(1) Weigh 2±0.05 g of crushed homogenate into 50 mL centrifuge tube, add 8 mL of **Ethyl acetate**, vortex for 30 s.

(2) Shake on shaker at 300 rpm for 20 minutes, centrifuge at 4000 r/min for 10 min at room temperature.

(3) Take 2 mL of supernatant to another centrifuge tube, dry with nitrogen evaporators or water bath at 50-60℃ (Please do it in a ventilated environment).

(4) Dissolve the residual with 2 mL of **N-hexane**, vortex for 10 s, add 1 mL of **Sample Diluent A** (Solution 5), vortex for 1 min.

(5) Centrifuge at 4000 r/min for 5 min at room temperature, discard the upper and middle liquid. Standing for 15min.

(6) Take 50 µL of lower layer liquid for analysis.

Note: Sample dilution factor: 2, Detection limit: 0.5 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. The unused ELISA Microtiter plate should be sealed as soon as possible 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 sealer, oscillate for 10 s gently to mix thoroughly, incubate for 30 min at 25℃.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 260 µL of **Wash Buffer** (Solution 9) to each well and wash. Repeat wash procedure for 4 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 100 µL of Substrate mixed solution to each well (**Substrate Reagent A** and **Substrate Reagent B** are fully mixed at ratio 1:1 by volume, the mixture should be used within 5 min, avoid using metal containers or stirring the reagents), Gently oscillate for 10 s to mix. thoroughly. Incubate at 25±2℃ for 15-20 min in shading light.
5. **Stop reaction:** add 50 µL of **Stop Solution** to each well, oscillate for 10s 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 5 min 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 form for accurate and fast analysis on a large number of samples.

Aflatoxin B1 (E-TO-E017) **Standard Curve**