AF (Total Aflatoxin) ELISA Kit

Catalog No: E-TO-E032

96T/96T*3

Version Number:	V1.9
Replace version:	V1.8
Revision Date:	2025.12.21

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 Total Aflatoxin (AF) 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, 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 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 AF. The concentration of AF 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±2℃, 20 min, 10-15 min.

Detection limit: Grain, Feed, Feed raw materials, Edible oil, Peanut meal, Coffee beans ---1.5 ppb.

Cross-reactivity: Aflatoxin B1 (AFB1) ---100.0%; AFB2---39.0%; AFG1---100.0%; AFG2---13.2%; AFM1---6.6%; AFM2--- < 1.0%; Other compounds--- < 0.1%.

Sample recovery rate: 100±20%.

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1.5 mL each (ppb=ng/mL=ng/g) (0 ppb, 1 ppb, 3 ppb, 9 ppb, 27 ppb, 81 ppb)
Antibody Working Solution	7 mL
HRP Conjugate	7 mL
Sample Diluent	50 mL
Sample Diluent B	50 mL
20×Concentrated Wash Buffer	25 mL
Substrate Reagent A	7 mL
Substrate Reagent B	7 mL
Stop Solution	7 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: Anhydrous ethanol (AR Grade), NaOH.

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℃.
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-E032. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other EE-TO-E032 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: **50% Ethanol-water Solution**

Anhydrous ethanol (V): deionized water (V) =1:1.

Solution 2: **Wash Buffer**

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

Solution 3: **1 M NaOH Solution**

Dissolve 40.01 g of NaOH to 1000 mL with deionized water, 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 feed, grain, feed raw materials sample:

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

(2) Weigh 5±0.05 g of crushed homogenate into 50 mL centrifuge tube, add 25 mL of **50% Ethanol-water Solution** (Solution 1), vortex for 5 min, centrifuge at 4000 r/min for 5 min at room temperature.

Note: For acidic samples with a PH value less than 5, the supernatant needs to be adjusted to neutral PH using **1 M NaOH Solution**, and then proceed with the next step.

(3) Take 100 µL of supernatant to another centrifuge tube, add 400 µL of **Sample Diluent**, mix fully.

(4) Take 50 µL for analysis.

Note: Sample dilution factor: 1, Detection limit: 1.5 ppb

Assay procedure

Restore all reagents and samples to room temperature (25±2℃) 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, add 50 μL of **HRP Conjugate**, then add 50 μL of **Antibody Working Solution** into each well. Gently oscillate for 10 s to mix thoroughly and cover the plate with sealer. Incubate for 20 min at 25±2℃ in shading light.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 260 μL of **Wash Buffer** (Solution 2) to each well and wash. Repeat wash procedure for 4 times, 15-30 s intervals/time. Invert the plate and pat it against thick clean with absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **Color Development:** add 100 μL mixture of **Substrate Reagent A** and **Substrate Reagent B** to each well (**Substrate Reagent A** and **Substrate Reagent B** are fully mixed at ratio of 1:1 according to 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 for 10-15 min at 25±2℃ in the dark (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 (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 solution or sample

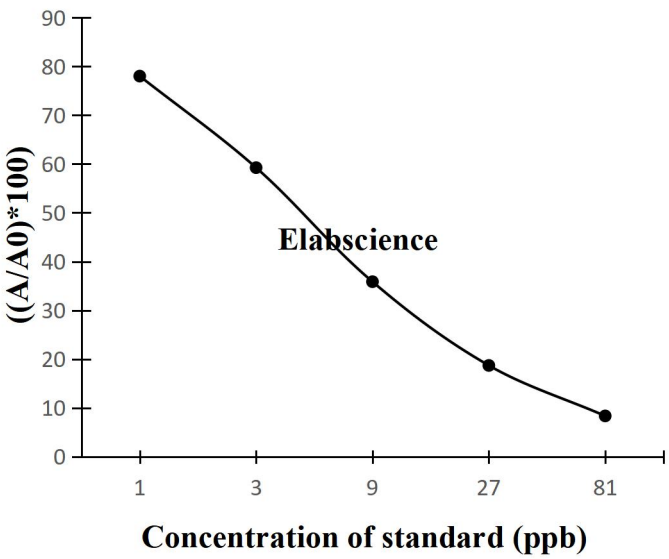
   A_0: Average absorbance of 0 ppb Standard solution

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 the average absorbance value 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.

Total Aflatoxin (E-TO-E032) **Standard Curve**



Supplementary Instruction Manual

Reagents: Acetonitrile (AR Grade), Methanol.

1. Solution preparation

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

Solution 4: **30% Methanol**

Methanol (V): deionized water (V) =3:7.

2. Sample pretreatment procedure

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

2.1 Pretreatment of peanut meal sample:

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

(2) Weigh 2±0.05 g of crushed homogenate into 50 mL centrifuge tube, add 15 mL **deionized water**, then add 5 mL **Acetonitrile**, vortex for 5 min, centrifuge at 4000 r/min for 5 min at room temperature.

(3) Take 200 µL of supernatant to another centrifuge tube, add 300 µL of **Sample Diluent** , mix fully.

(4) Take 50 µL for analysis.

Note: Sample dilution factor: 1, Detection limit: 1.5 ppb

2.2 Pretreatment of edible oil sample:

(1) Take 1±0.05 g of sample into 10 mL centrifuge tube, add 5 mL of **30% Methanol** (Solution 4), vortex for 1 min.

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

(3) Take 100 µL of the lower clear liquid to another centrifuge tube, add 400 µL of **Sample Diluent B**, mix fully

(4) Take 50 µL for analysis.

Note: Sample dilution factor: 1, Detection limit: 1.5 ppb

2.3 Pretreatment of Coffee beans sample:

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

(2) Weigh 5±0.05 g of crushed homogenate into 50 mL centrifuge tube, add 25 mL of **Acetonitrile**, vortex for 5 min, centrifuge at 4000 r/min for 5 min at room temperature. Note: For acidic samples with a PH value less than 5, the supernatant needs to be adjusted to neutral PH using **1 M NaOH Solution**, and then proceed with the next step.

(3) Take 100 µL of supernatant to another centrifuge tube, add 400 µL of **Sample Diluent**, mix fully.

(4) Take 50 µL for analysis.

Note: Sample dilution factor: 1, Detection limit: 1.5 ppb

Assay procedure

Consistent with the main manual.