# Elabscience®

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## FB1(Fumonisin B1) ELISA Kit

Catalog No: E-TO-E005

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 fumonisin B1 (FB1) 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 FB1. During the reaction, FB1 in the samples or standard competes with FB1on the solid phase supporter for sites of FB1 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 FB1. The concentration of FB1 in the samples can be calculated by comparing the OD of the samples to the standard curve.

## Technical indicator

**Sensitivity:** 0.5 ppb (ng/mL)

**Reaction mode:** 25℃, 30min~15min

**Detection limit:** Corn, feed---50ppb,Edibleoil---10ppb

**Cross-reactivity:**Fumonisin B1(FB1) ---100%

**Sample recovery rate:**  Feed---95%±15%, Corn---100%±15%, Edibleoil---85%±15%

## Kits components

| Item | Specifications |
|---|---|
| Micro ELISA Plate | 96 wells |
| Standard Liquid(black cap) | 1mL each:<br>(0ppb,0.5ppb,1.5ppb,4.5ppb,13.5ppb,40.5ppb) |
| 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 |
| 10×ConcentratedReconstitution fluid (yellow cap) | 50mL |
| 20×Concentrated Wash Buffer(white cap) | 40mL |
| Product Description | 1 copy |

## Other supplies required

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

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

**Reagent:**Methanol.

## 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 extract solution):70%Methanol. Methanol: Deionized water (Volume) =70:30. Solution2: (Reconstitution solution): for sample re-dissolution. Dilute the 10×concentratedreconstitution solution withdeionized water (1:9). Store at 4℃, valid for 1 month. Solution 3: (wash working buffer): dilute the 20× concentrated wash buffer with deionized water (1:19).

3. **Sample pretreatmentprocedure**

**3.1 Pretreatment of corn, feed:**

(1) Weigh1g of crushed homogenate into the 50mL EP tube, add 5mLof 70% methanol, oscillate for 5min. Centrifuge at 4000r/min for 10min at room temperature;

(2) Take 0.1mLof supernatant, add 1.9mLof Reconstitution fluid, oscillate for 2min;

(3) Take 50μL for detection and analysis.

   **Note: Sample dilution factor: 100, minimum detection dose: 50ppb**

   If fumonisins B1 content is higher in the sample, take the mixed liquid from step 2, increase the dilution times and diluted with Reconstitution fluid, the sample dilution multiple is the actual dilution multiple at the moment.

**3.2 Pretreatment of edible oil:**

(1) Take5mLof sample into the 50mL EP tube, add 8mL of n-hexane and 5mL of 70%methanol, oscillate for 5min, centrifuge at 4000r/min for 10min at room temperature.

(2) Discard the supernatant, take 100μLof under layer liquid, and add1.9of Reconstitution fluid, mix.

(3) Take 50μL for detection and analysis.

   **Note: Sample dilution factor: 20, minimum detection dose: 10ppb**

## 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 mLwash working buffer with deionized or distilled water before experiment.

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 for 15min at 25℃ in dark.

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 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 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 effect 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.