Technical Manual

AFB1(Aflatoxin B1) ELISA Kit

- **Catalogue Code: FSES0075**
- **Competitive ELISA Kit**
- **Research Use Only**

Contents

1. Key features and Sample Types

Sensitivity:

0.03 ppb (ng/mL)

Assay Procedure:

25°C, 30 min~ 15 min

Detection Limit:

Cereals - 0.15 ppb; Corn skin, Wheat bran - 0.6 ppb; Edible oil, Peanut - 0.6 ppb,
Biscuits - 0.3 ppb; Beer - 0.3 ppb; Wine, Soy sauce, Vinegar - 0.15 ppb

Cross Reactivity:

Aflatoxin B1 (AFB1) - 100%

Sample Recovery rate:

Cereals, Corn skin, Wheat bran, Edible oil - 85±15%; Peanut - 82±15%;
Biscuit - 83%±15%; Beer - 84%±15%;
Wine, Soy sauce, Vinegar - 87%±15%

Storage:

2-8°C for 6 months.

Expiry:

See Kit Label

2. Storage

Store the kit at 2~8°C. 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°C.

3. Test Principle

This kit uses a Competitive-ELISA method. It can detect Aflatoxin B1 (AFB1) in samples, such as Cereals, formula feed, edible oil, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody working solution, standard 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.

4. Kit Contents

Each kit contains reagents for 96 assays including:

No.	Component	96-WellKit
1	ELISA Microtiter plate	96 wells
2	Standards	1mL each (0 ppb, 0.03 ppb, 0.06 ppb, 0.12 ppb, 0.24 ppb, 0.48 ppb)
3	HRP Conjugate	5.5 mL
4	Antibody Working Solution	5.5 mL
5	Substrate Reagent A	6 mL
6	Substrate Reagent B	6 mL
7	Stop Solution	6 mL
8	20×Concentrated Wash Buffer	40 mL
9	Plate Sealer	3 pieces
10	Sealed Bag	1 piece
11	Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Additional materials required:

Other materials required but not supplied

- **Instrument:** Microtiter plate reader, Printer, Homogenizer, Nitrogen Evaporators, Water bath, Vortex mixer, Centrifuge, Graduated pipette, Balance (sensitivity 0.01g).

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

- **Reagents:** Methanol, N-hexane, Trichloromethane or Dichloromethane.

5. Experimental Preparation

Bring 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 pre-treatment Notice:

Experimental apparatus should be clean, and the pipette should be disposable to avoid cross-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: 70% Methanol

Methanol (V): Deionized water (V) =7:3

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: 35% Methanol

70% Methanol (V): Deionized water (V) =1:1

3. Sample pre-treatment procedure

Targets may be distributed unevenly, resulting in no detection. To avoid this, ensure to take sufficient samples when sampling.

3.1 Pre-treatment of cereals sample:

1. Homogenize the sample, use Homogenizer.
2. Weigh 2 g of homogenate sample into 50 mL centrifuge tube, add 5 mL of **70% Methanol** (Solution 1), vortex for 5min, centrifuge at 4000 rpm for 10 min at room temperature;
3. Take 0.5 mL of supernatant to another centrifuge tube, add 0.5 mL of deionized water, mix;
4. Take 50 μL for detection and analysis.

Note: Sample dilution factor: 5, detection limit: 0.15 ppb

3.2 Pre-treatment of corn skin, wheat bran sample:

1. Homogenize the sample, use Homogenizer.
2. Weigh 2 g of homogenate sample into 50 mL centrifuge tube, add 20 mL of **70% Methanol** (Solution 1), vortex for 5 min, centrifuge at 4000 r/min for 10 min at room temperature.
3. Take 0.5 mL of supernatant to another centrifuge tube, add 0.5 mL of deionized water. Mix fully.
4. Take 50 μL for detection and analysis.

Note: Sample dilution factor: 20, detection limit: 0.6 ppb

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

3.3 Pre-treatment of edible oil, peanut sample:

1. Homogenize the sample, use Homogenizer.
2. Weigh 2 mL/2g of homogenate sample into 50 mL centrifuge tube, add 8 mL of **N-hexane** and 10 mL of **70% Methanol** (Solution 1), vortex for 5 min, centrifuge at 4000 r/min for 10 min at room temperature;
3. Discard the upper liquid, and take 0.5 mL of lower liquid to another centrifuge tube, add 0.5 mL of deionized water, mix fully (liquid A);
4. Take 0.5 mL of liquid A from step 3, then add 0.5 mL of **35% Methanol (**Solution 3**),** vortex for 30 s;
5. Take 50 µL for detection and analysis.
   Note: Sample dilution factor: 20, detection limit: 0.6 ppb

3.4 Pre-treatment of biscuits sample:

1. Homogenize the sample use Homogenizer.
2. Weigh 2 g of homogenate sample into 50 mL centrifuge tube, add 10 mL of **70% Methanol** (Solution 1), vortex for 5 min, centrifuge at 4000 r/min for 10 min at room temperature;
3. Take 2 mL of supernatant to another centrifuge tube, add 4 mL of **Trichloromethane or Dichloromethane**, vortex for 5 min, centrifuge at 4000 r/min for 10 min at room temperature;
4. Take the upper liquid to another centrifuge tube, keep the lower liquid for use (lower liquid A). Add 4 mL of **Trichloromethane or Dichloromethane** to the upper liquid, vortex sufficiently for 5 min, centrifuge at 4000 r/min for 10 min at room temperature. Discard the upper liquid and keep the lower liquid (lower liquid B);
5. Mix lower liquid A and lower liquid B thoroughly;
6. Take 2 mL of mixed lower liquid to another centrifuge tube and dry with nitrogen evaporators/water bath at 50-60°C;
7. Add 0.5 mL of **70% Methanol** (Solution 1) to dried materials to dissolve thoroughly, add 0.5 mL of deionized water mix fully.
8. Take 50 µL for detection and analysis.
   Note: Sample dilution factor: 10, detection limit: 0.3 ppb

3.5 Pre-treatment of beer sample:

1. Stir beer thoroughly to remove CO2, take 2 mL of beer sample and add 1 mL of deionized water, then add 7 mL of **Methanol**, vortex for 5 min.
2. Take 0.5 mL of mixed sample liquid and add 0.5 mL of deionized water to another centrifuge tube, mix fully.
3. Take 50 µL for detection and analysis.
   Note: Sample dilution factor: 10, detection limit: 0.3 ppb

3.6 Pre-treatment of wine, soy sauce, vinegar sample:

1. Take 2 mL of sample and add 2 mL of deionized water, then add 10 mL of **Trichloromethane or Dichloromethane**, vortex for 5 min, centrifuge at 4000 r/min for 10 min at room temperature.
2. Take 1 mL of lower liquid to another centrifuge tube and dry with nitrogen evaporators or water bath at 50-60°C.
3. Add 0.5 mL of **70% Methanol** (Solution 1) to dried materials to dissolve thoroughly, add 0.5 mL of deionized water, mix fully;
4. Take 50 µL for detection and analysis.

Note: Sample dilution factor: 5, detection limit: 0.15 ppb

6. Assay Procedure

Bring all reagents and samples to room temperature (25°C) 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°C.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. **Standard and Samples must be tested 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 plate sealer. vortex for 5s gently to mix thoroughly, incubate at 25°C for 30 min away from direct sunlight.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 300 µL of **Wash Buffer (**Solution 2) to each well and wash. Repeat wash procedure for 5 times, 30s intervals/time. Invert the plate and pat it against absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **Colour Development:** add 50 µL of **Substrate Reagent A** to each well, and then add 50 µL of **Substrate Reagent B**. Gently vortex for 5s to mix thoroughly. Incubate at 25°C for 15 min away from direct sunlight (The reaction time may be shortened or prolonged according to the depth of the colour).
5. **Stop reaction:** add 50 µL of **Stop Solution** to each well, vortex 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 10 min after stop reaction.

7. Data 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 form for accurate and fast analysis on a large number of samples.

8. 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°C.
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. ELISA Microtiter plate should be covered by plate sealer. Avoid the kit to strong light.
5. **Each reagent is optimized for use in the FSES0075. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other FSES0075 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns colour. When OD value of standard (concentration: 0) < 0.5 unit (A450nm < 0.5), it indicates the reagents are 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. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
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.

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

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