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Aflatoxin B1 (AFB1) Enzyme Linked Immunoassay (ELISA) Kit

Product Number: ELK028

Shipping and Storage

1. The reagent kit should be stored at 2-8℃ and should not be frozen.
2. Unused microplates should be sealed and stored dry.

Component

Component	96T
Detachable enzyme-linked immunosorbent assay (ELISA) plates pre coated with AFB1 conjugated antigen	1 plate(12 holes×8 strips)
AFB1 standard	6 bottles (1mL/bottle)
Anti-AFB1 antibody enzyme conjugate	6mL
Chromogen solution A	6mL
Chromogen solution B	6mL
Stop solution	6mL
Sample diluent	(10×, 6mL)
Concentrated wash buffer	(30×, 6mL)

Description

This kit uses a direct competitive ELISA method, in which aflatoxin B1 (AFB1) conjugated antigen is coated on a microplate, and aflatoxin B1 (AFB1) standard or sample is added. Free aflatoxin B1 (AFB1) competes with pre coated aflatoxin B1 (AFB1) conjugated antigen on the microplate for anti aflatoxin B1 (AFB1) antibody enzyme labeling. TMB substrate is used for color development, and the color changes from blue to yellow after adding termination solution. The absorbance value is inversely proportional to the content of aflatoxin B1 (AFB1) in the sample, and the content of aflatoxin B1 (AFB1) in the sample is calculated by the standard curve.

Application

This reagent kit is used to detect the content of aflatoxin B1 (AFB1) in soybean flour, feed and grains, corn and food, fish, shrimp and meat tissues (such as chicken, beef and pork), eggs, honey, milk, goat milk, serum and urine samples, and cells.

Materials required but not supplied

1. Equipment: 450nm wavelength enzyme-linked immunosorbent assay (ELISA) reader, pulverizer, graduated cylinder, oscillator, funnel, Whatman No 1 or equivalent filter paper, micropipette.
2. Reagents: Deionized water or distilled water, Methanol.

Note

1. Please read the instructions carefully before using the kit.
2. Do not use expired kits.
3. Before use, bring all reagents to room temperature (25±2°C). It is recommended to equilibrate for at least 2 hours.
4. The standard contains aflatoxin B1 (AFB1), so special attention should be paid when using it and gloves should be worn during operation.
5. The stop solution contains sulfuric acid. Prevent skin burns and corrosion of clothing.
6. Do not reuse pipette tips for different standards or samples, as this may affect the test results.
7. Do not mix reagents from different kit lots; do not reuse pipette tips for different standards or samples, as this may affect the

test results.

8. The sample diluent provided in this kit must be used for diluting samples; otherwise, results may be affected.
9. Avoid generating bubbles when mixing reagents.

Reagent Preparation

1. AFB1 standard solution: 0ng/mL, 0.1ng/mL, 0.3ng/mL, 0.9ng/mL, 2.7ng/mL, 8.1ng/mL.
2. Concentrated wash buffer: Dilute 1:30 (1+29) with distilled water before use.
3. Sample diluent: Dilute 1:10 (1+9) with distilled water before use.
4. Chromogen solution: Ready for use, avoid direct light exposure.
5. Stop solution: Ready to use.

Sample Processing Procedure

Strictly follow the instructions during sample extraction. Accurate dilution during extraction is crucial; otherwise, results may be inaccurate. Store samples in a cool, dark place and under refrigeration.

1. Take 10g of homogenized sample, add 20mL of 70% Methanol solution.
2. Vigorously shake for 3 minutes.
3. Filter using Whatman No. 1 filter paper.
4. Take 25μL of the processed sample and add 25μL of Sample Diluent to the reaction well (sample dilution factor is 2).

Protocol

1. Notes before Assay

1.1. Before starting the experiment, equilibrate all reagents outside the box to room temperature (25±2°C) for approximately 2 hours. Remove the required microplate strips only after equilibration. Immediately reseal and store any unused strips dry at 2~8°C.

Note: Ensure thorough equilibration, otherwise detection precision and accuracy will be affected.

1.2. Return reagents to 2~8°C immediately after use.

1.3. Do not modify the assay procedure.

1.4. Use precise micropipettes.

1.5. Once the operation starts, do not interrupt any step.

1.6. The reproducibility of ELISA results depends heavily on the operational procedure; please follow the requirements strictly.

1.7. To avoid cross-contamination, use separate pipette tips for each standard and sample.

1.8. When adding solutions, do not let the pipette tip touch the liquid or inner surface of the well.

2. Assay Procedure

2.1. Pre-label the positions for B0, standards, and samples. Duplicate wells are recommended.

2.2. Remove the required number of microplate strips (removable). Reseal the unused strips and immediately return them to 2~8°C storage.

2.3. Dilute the Sample Diluent (10×) and Concentrated Wash Buffer (30×) to working solutions using distilled or deionized water.

2.4. Add 50μL of the 0.0ppb standard solution to the B0 well(s).

2.5. Add 50μL of each standard solution to the respective standard wells.

2.6. Add 50μL of each sample solution to the respective sample wells.

2.7. Add 50μL of anti-AFB1 antibody enzyme conjugate to all wells.

2.8. Gently shake the reaction plate for a few seconds.

3. Incubate at 37°C for 30 min (Gently tap the plate intermittently during incubation to reduce well-to-well variation).

Discard the liquid from the wells. Wash the microplate 5 times with Wash Buffer. After the last wash, blot the plate firmly onto

absorbent paper to remove residual liquid.

4. Reaction

4.1. Immediately after the washing step, add 50µL of Chromogen Solution A to each well, followed by 50µL of Chromogen Solution B, using a micropipette. Gently shake the plate to mix thoroughly.

4.2. Incubate at 37°C for 10 min.

4.3. Add 50µL of Stop Solution to each well and mix.

4.4. Measure the absorbance at 450 nm. Read the results within 5 minutes.

Result judgment

1. Quantitative Analysis

1.1. Calculate the average absorbance (B) for each standard and sample. Divide this value (B) by the average absorbance of the zero standard (B0) and multiply by 100% to obtain the Percent Absorbance (%B/B0).

B = Average absorbance of standard or sample solution

B0 = Average absorbance of the 0 ppb standard solution

1.2. Draw a standard curve graph with the logarithm of AFB1 concentration as the X-axis and the percentage absorbance value as the Y-axis. Based on the percentage absorbance value of the sample, the abscissa of the corresponding point can be obtained from the curve, which is the logarithm of the AFB1 concentration. The logarithm obtained is the AFB1 concentration C (ng/mL) in the measuring solution.

1.3. As the sample was pre-diluted, the concentration obtained from the standard curve must be multiplied by the corresponding dilution factor.

2. Semi-Quantitative Determination

2.1. Visual Semi-Quantitative Determination: Select an appropriate standard to run concurrently with the samples. Compare the color intensity of the sample with that of the standard to determine if the sample concentration is less than or greater than the standard value.

2.2. Instrumental Semi-Quantitative Determination: Select an appropriate standard to run concurrently with the samples. Compare the absorbance value of the sample with that of the standard to determine if the sample concentration is less than or greater than the standard value.

Specificity

Substance	Cross-Reactivity
Aflatoxin B1	100%
Aflatoxin B2	80%
Aflatoxin G1	75%
Aflatoxin G2	30%

Kit Parameters

1. The detection limit of this kit is 0.05ng/mL.
2. The optimal absorbance value for B0 should be greater than 1.0.
3. The intra-assay Coefficient of Variation (CV) is less than 8%, and the inter-assay CV is less than 15%.
4. The recovery rate using the tissue sample extraction method provided in this manual is greater than 80%.
5. The standard curve range provided by the kit is 0.1ng/mL~8.1ng/mL.

Analysis Limitations

Samples testing positive with this kit should be confirmed by an alternative method, such as HPLC or GC/MS.