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Datasheet

Ver.1 Date : 20180222

AFB1 (Aflatoxin B1) ELISA Kit

Cat# E0034– 96 well

Storage at 4°C for 1 year. Avoid freeze

TEST PRINCIPLE

This kit uses Competitive-ELISA as the method. It can detect Aflatoxin B1 (AFB1) in samples, such as grain, 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.

SPECIFICITY

- Sensitivity: 0.03 ppb (ng/mL)
- Reaction mode: 25°C, 30-15 min
- Detection limit:
  Grain---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:
  Grain, Corn skin, Wheat bran, Edible oil---85±15%; Peanut---82±15%;
  Biscuits---83%±15%; Beer---84%±15%;
  Wine, Soy sauce, Vinegar---87%±15%

CONTENTS & STORAGE

Component	Specifications
Micro ELISA Plate	96 wells
Standard Liquid	1mL each (0 ppb, 0.03 ppb, 0.06 ppb, 0.12 ppb, 0.24 ppb, 0.48 ppb)
HRP Conjugate	5.5 mL
Antibody Working Solution	5.5 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
20× Concentrated Wash Buffer	40 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

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

OTHER METERIALS 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:** Methanol, N-hexane, Trichloromethane or Dichloromethane.

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 cross-contamination during the experiment.
2. **Solution preparation**
   - 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 pretreatment procedure**

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

   3.1 Pretreatment of grain 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), oscillate 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, minimum detection limit: 0.15 ppb

   3.2 Pretreatment 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)**, oscillate 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. Take 50 µL for detection and analysis.

   Note: Sample dilution factor: 20, minimum 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 Pretreatment of edible oil, peanut sample

   (1) Homogenize the sample, use Homogenizer.

   (2) Weigh 2 g of homogenate sample into 50 mL centrifuge tube, add 8 mL of **N-hexane** and 10 mL of **70% Methanol (Solution 1)**, oscillate 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), oscillate for 30 s;

(5) Take 50 µL for detection and analysis.

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

3.4 Pretreatment 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)**, oscillate 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**, oscillate 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, oscillate 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℃;

(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, minimum detection limit: 0.3 ppb

3.5 Pretreatment 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**, oscillate 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, minimum detection limit: 0.3 ppb

3.6 Pretreatment 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**, oscillate 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℃.

(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, minimum detection limit: 0.15 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 plate sealer. oscillate for 5s gently to mix thoroughly, incubate at 25℃ for 30 min in shading light.
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 thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **Color Development:** add 50 µL of **Substrate Reagent A** to each well, and then add 50 µL of **Substrate Reagent B**. Gently oscillate for 5s to mix thoroughly. Incubate at 25℃ for 15 min in shading light (The reaction time may be shortened or prolonged according to the depth of the color).
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 10 min after stop reaction.

RESULT ANALYSIS

1. **Absorbance (%) =A/A0×100%**

   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.

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. ELISA Microtiter plate should be covered by plate sealer. Avoid the kit to strong light.
5. **Each reagent is optimized for use in the E0034. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E0034 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns color. When OD value of standard (concentration: 0) ＜0.5 unit (A450nm＜0.5), it indicates the reagent may be 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.

STORAGE and VALIED PERIOD

Store at 2~8℃ for 1 year. Avoid freeze.

Please store the opened kit at 2~8℃, the shelf life of the opened kit is up to 1 month.

Expiry date: expiration date is on the packing box.