Total Aflatoxin ELISA Kit

A、Summary

Aflatoxins are toxic and potent human carcinogen, which may contribute to human liver cancer. They are metabolites of the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. There are four principle types of aflatoxin: B1, B2, G1 and G2, which are named for their respective innate fluorescent properties. Aflatoxins can be found mainly in cereals, nuts, cotton seeds.

B、Use Principles

The kit is an indirect competitive enzyme-labeled immunoassay. The aflatoxin antigen is precoated on the wells. The precoated antigen compete the aflatoxin antibody (AFT antibody solution) with aflatoxin in the sample, anti-aflatoxin antibody binds to the aflatoxin-HRP enzyme conjugate. Then pipe the substrate solution to the wells to convert the color. The color of unknown samples is compared to the color of the standard and the aflatoxin concentration of the samples is derived.

C、Intended Use

The Total Aflatoxin ELISA Kit is a competitive ELISA for the qualitative or quantitative analysis of aflatoxin in grain, peanut & peanut butter, feed and feed material.

D、Materials Required But Not Provided

1、 Devices:

(1) Microwell plate Reader (450 nm/630 nm)
(2) Oscillator/ Vortex
(3) Thermostat incubator
(4) Centrifuge (or quantitative filter)
(5) Scales (sensibility: 0.01g)
(6) Pipette: 50 mL
(7) Triangular flask (with stopper): 100 mL
(8) Centrifuge tube: 15 mL
(9) Micropipette:
Single channel pipet
(10 μL-100 μL、100 μL-1000 μL)
Multi-channel pipet
(30 μL-300 μL)

2、 Reagent:

(1) Methanol (AR)
(2) Deionized water

E、Provided Materials and Reagent

Item	Components	Specification
1	Microtiter plate (96 wells)	1 pcs
2	Standards (0 ppb, 0.06 ppb, 0.2 ppb, 0.6 ppb and 2 ppb)	1 mL /each
3	AFT Enzyme Conjugate	12 mL
4	AFT Antibody Solution	12 mL
5	Substrate Solution	10 mL×2
6	Stop Solution	6 mL
7	10× Conc. Washing Solution	40 mL
8	Package Insert	1 pcs
9	Plate Cover Sheet	2 pcs
10	Testing Report	1 pcs

F、Solution preparation

1、 60% methanol solution preparation:
Methanol and distilled/deionized water V:V=3:2 mix together.

2、 10% methanol solution preparation:
Methanol and distilled/deionized water V:V=1:9 mix together.

3、 Wash buffer preparation:
Dilute the 10× Conc. Washing Solution with distilled or deionized water (volume ratio between the water and the washing solution is 9:1). The dilution can be stored 1 month at 4℃.

G、Sample Preparation：

Grain, Peanut and Peanut butter、Feed and Feed material：

1、 Weigh 5.0 g homogenized sample to a 100 mL triangular flask with stopper.
2、 Add 25 mL of 60% methanol solution.
3、 Blend vigorously for 10 minutes on an oscillator (150 r/min) or vortex for 5 minutes.
4、 Take solution to a tube and centrifuge for 5 minutes at 4,000 r/min (or let it stand for 3 minutes and filter it by quantitative filter paper).
5、 Transfer 1 mL top layer liquid or filtrate to a new tube and add 4 mL of deionized water.
6、 Blend for 5 second and take 50 μL mix solutions for assay.

Dilution factor: 25

H、Assay Procedure

1、 Take out the reagents from the refrigerator and allow them to reach room temperature (20-25℃) prior to running the test (about 0.5~1 hour).

2、 Place the appropriate number of test wells into a microwell holder. Be sure to re-seal unused wells in the zip-lock bag with desiccant stored under the temperature 2-8℃ **(Do not freeze)**.

3、 Numbering: Number the microwells for the standards and samples separately. Running standards and samples in duplicate.

4、 Using a pipette with disposable tips, add 50 µL of Standards and Samples to the appropriate test wells. Be sure to use a clean pipet tip for each. Then dispense 50 µL of AFT Enzyme Conjugate into each test well. At last, dispense 50 µL of AFT Antibody Solution (please use multi-channel pipet) into each test well.

5、 Shake the plate gently to mix contents, cover the plate rack and incubate the test wells at 25℃ keeping away from light for 15 minutes.

6、 Open the cover on the plate and dump the contents of the wells into an appropriate waste container. Fill the wells to overflowing with Wash buffer and dump (250 µL each well). Repeat for a total of four washes (wait 10 seconds between each wash).

7、 Following the last wash, tap the inverted wells onto absorbent paper to remove the residue of the wash buffer.

8、 Dispense 100 µL of Substrate into each well in order (please use multi-channel pipet).

9、 Shake the plate gently. Cover the plate rack and incubate the wells at 25℃ for 5 minutes.

10、 Dispense 50 µL of Stop solution into each test well (please use multi-channel pipet). Shake the plate rack gently to mix.

11、 Read and record the absorbance of the wells at 450 nm using a plate reader (dual wavelength 450/630 nm detecting is advised) within 5 minutes.

I、Result Calculation

Method I：

Analyze the result by using the software (Ridasoft)

Note: If the concentration of Total aflatoxin in the sample exceeds 50 ppb, please dilute the mix solution using 10% methanol solution. Take the diluted solution for assay; please consider the extra diluting factor when calculating the result.

Method II：

$$\frac{\text{Absorbance standard (or sample)}}{\text{Absorbance (0 ppb standard)}} \times 100 = \frac{B}{B0}$$

Use Excel to convert the standard concentration to Log and B/B0, and then make the linear regression to get the concentration (equation as above).

B：the average absorbance of STD or test sample

B0：the average absorbance of STD 0 ppb

J、Detection Limit:

The lowest concentration standard of Vaccigen AFT ELISA is 0.06 ppb. The absorbance value of this concentration is significantly different from the absorbance value of the negative standard solution (0 ppb).

K、Standard curve

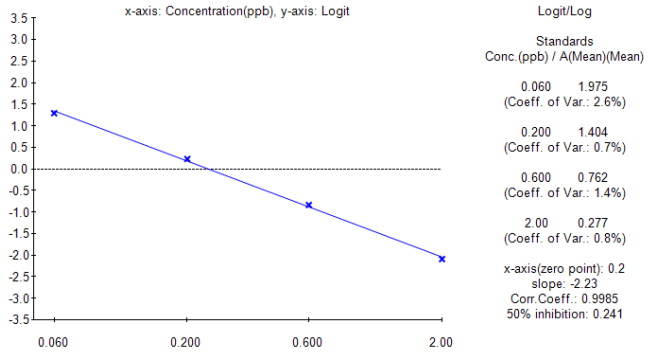

Fig.1：Calibration Curve for VACCIGEN AFT ELISA Kit

L、Precision (CV %): intra-lab assay: CV%≤10%

For the precision test of the AFT ELISA kit, under the sampling number = 6, the coefficient of variation (CV%) of the absorbance values of the standard solutions with different concentrations is shown in the figure below, showing that the AFT ELISA kit has high reproducibility:

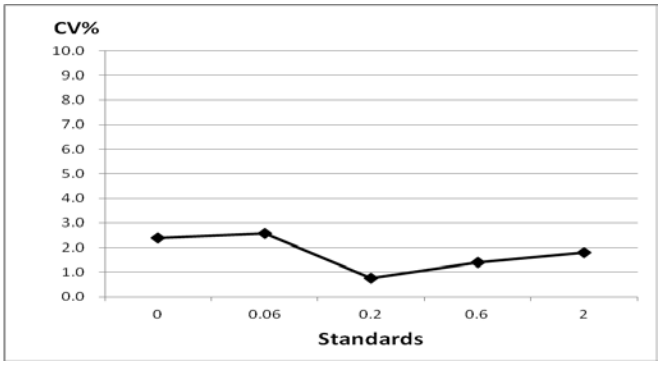

Fig.2：Interassay Precision Profile for VACCIGEN AFT ELISA Kit

M、Sensitivity

Sample	ppb
Grain、Peanut、Peanut butter、Feed and Feed Material	1.5

N、Recovery rate

Sample	%
Grain、Peanut、Peanut butter、Feed and Feed Material	70~130

O、Cross-Reactivity

Aflatoxin types	%
AFB1	100
AFB2	50
AFG1	40
AFG2	10
AFM1	20
AFM2	<1

P、Precautions

1、 Reagents should be brought to room temperature, 20 – 25℃ prior to use. Avoid prolonged (> 24 hours) storage at room temperature.

2、 Immediately start the next step of operation after the washing procedure, avoid the microwell being dry from affecting the detecting result.

3、 Shake the reagent before use.

4、 The Stop Solution is 2M sulfuric acid. Avoid contacting with skin and mucous membranes. Immediately clean up any spills and wash area with large amounts of water. If it is occurred, immediately flush with large amounts of water.

5、 Do not use reagents after expiration date. Do not substitute reagents from any other manufacturer into the kit. Do not combine reagents from other Aflatoxin ELISA Kits with different Lot numbers.

6、 The kit should be store under the temperature 2-8℃, do not freeze. Be sure to re-seal unused wells in the zip-lock bag with desiccant stored. Avoid directly exposure of the reagents to light.

7、 Any color conversion indicates that the reagent is deteriorated, please drop it. If the absorbance of negative control absorbance is less than 0.5, the reagent is probably deteriorated.

8、 The incubating time is usually 5 minutes after dispensing Substrate into the wells. If the color is too light, please prolong the incubating time but should not exceed 7 minutes. Conversely, reduce the incubating time.

9、 Crystallization of the Concentrated Wash buffer is a normal phenomenon, heat it before using.

10、 The optimum reaction temperature is 25℃, too high or too low temperature would induce the change of the absorbance and sensitivity.