Total Aflatoxin (AFT) ELISA Kit

Technical Manual

(ELISA)

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1 Principle and Application

Aflatoxins are secondary metabolites produced by fungi of the Aspergillus species, including Aspergillus flavus and Aspergillus parasiticus. They primarily contaminate crops such as corn, peanuts, and nuts. Aflatoxins are mainly categorized into four types: AFB1, AFB2, AFG1, and AFG2. The total amount of aflatoxins generally refers to the combined quantity of these four toxins. They are classified as Group I carcinogens, demonstrated to pose significant hazards to organs and tissues such as the liver and kidneys in humans and animals. Aflatoxins are potent and highly toxic carcinogenic substances.

This kit adopts the method of indirect competitive enzyme-linked immunoassay (ELISA) to detect Aflatoxins(AFT) in the sample such as grains and feed. The kit is composed of Microtiter Plate coated with coupled antigens, HRP conjugate, antibodies, standards and other supporting reagents. During the detection, with adding standards or samples, the AFT in the samples will compete with the coupled antigens to combine with anti-AFT antibodies. After adding HRP conjugates, take coloration with TMB substrates. Absorbance value of the samples is a negative correlation with AFT content. Lastly, comparing with the standard curve, the obtained concentration is multiplied by the sample dilution ratio. AFT residues in sample can be concluded.

2 Technique Data

2.1 Kit Sensitivity: 0.02ppb (ng/mL)

2.2 Reactive Mode: 25°C, 30min ～ 15min

2.3 Detection Limits:

Sample	Detection Limits
Grain	0.1ppb
Feed	0.2ppb

2.4 Cross-reaction Rate:

Aflatoxin B1 ..... 100%
Aflatoxin B2 ..... 80%
Aflatoxin G1 ..... 75%
Aflatoxin G2 ..... 45%
Aflatoxin M1 ..... 8%

2.5 Sample Recovery Rate:

Sample	Recovery Rate
Grains and feed	85±15%

3 Composition of the Kit

Reagent	Specification
Microtiter Plate	8wells× 12strips
Standard: 0ppb, 0.02ppb, 0.04ppb, 0.08ppb, 0.16ppb, 0.32ppb (black cap)	1.0mL each
High Standard (black cap)： 100ppb	1×1.0mL
Antibody solution (blue cap)	1×5.5mL
HRP conjugate (red cap)	1×5.5mL
Substrate Reagent A (white cap)	1×6mL
Substrate Reagent B (black cap)	1×6mL
Stop Solution (yellow cap)	1×6mL
Concentrated Wash Buffer (20×)(white cap)	1×40mL
Instruction	1
Adhesive Membrane	1
Sealed bag	1

4 Materials Required but Not Supplied

4.1 Equipment: microplate reader, printer, grinder (for homogenizing solid samples), vortex mixer (for shake and mix), centrifuge, graduated transfer pipette, and balance with a division value of 0.01 g, constant temperature device(25°C);

4.2 Micropipette: single-channel (20-200μL and 100-1000μL), and multi-channel 300μL;

4.3 Reagents: methanol.

5 Experimental preparation

Restore all reagents and samples to room temperature (adjust to around 25°C) for more than 30 min before use. This is a crucial step to ensure there is no precipitation in the reagents.

5.1 Notice Before Sample Processing:

Please note that the labware must be clean. Use disposable pipette tips to avoid contamination of interference results.

5.2 Solution preparation:

Solution 1: Sample Extraction Solution

70% Methanol solution, (Methanol/Deionized water= 7: 3).

Solution 2: Working Wash Buffer

Dilute the concentrated wash buffer (20×) by a factor

of 20 (Concentrated wash buffer/Deionized water= 1: 19).

5.3 Sample pretreatment steps:

5.3.1 Grain treatment.

1) Weigh 2g±0.05g of homogenized samples into a 50mL centrifuge tube, pipette 5mL of Sample Extraction Solution (Solution 1), shake them for 5min and centrifuge at 4000 rpm at room temperature for 10min.

2) Take 0.5mL of supernatant, add 0.5mL of deionized water, and mix fully.

3) Take out 50μL for test.

Dilution times of the sample:5 Detection limits: 0.1ppb

5.3.2 Feeds treatment.

1) Weigh 2g±0.05g of homogenized samples into a 50mL centrifuge tube, pipette 10mL of Sample Extraction Solution (Solution 1), shake them for 5min and centrifuge them at 4000 rpm at room temperature for 10min.

2) Take 0.5mL of supernatant, add 0.5mL of deionized water, and mix fully.

3) Take out 50μL for test.

Dilution times of the sample:10 Detection limits: 0.2ppb

Note: If the content of Aflatoxin in the sample is relatively high, take the mixed liquid from step 2) and dilute it with 35% methanol. The dilution factor at this point becomes the actual dilution factor. For example, if the liquid from step 2) is diluted 10 times with 35% methanol, the actual dilution factor is 10×10=100. The detection limit is 2 ppb.

6 ELISA procedure

Place all reagents and samples to room temperature (adjust to around 25°C) for 30min. Gently shake the reagent bottles before use.

Take out the frame of the microplate along with the required number of wells. Then place the unused microplate wells into the sealed bag with the desiccant provided. Store the remaining kit in the refrigerator at 2-8°C.

Step 1: Number: Number the samples and standard corresponding microwells in order, make 2-well parallel trials for each sample and standard, and record the locations of sample wells and standard wells.

Step 2: Incubation: Add 50μL of standard or sample into each numbered well, then add 50μL of HRP conjugate per well. Next, add 50μL of antibody solution into each well. Finally, cover the Microtiter Plate with the adhesive membrane, shake gently by hand (or use a microplate shaker) for 5s and incubate for 30 min at 25°C.

Step 3: Washing: Uncover the adhesive membrane carefully, discard liquid in the wells, pipette 350μL of Working Wash Buffer (Solution 2) to every well, let stand for 30 seconds then drain, repeat 5 times. Invert the plate and tap it against a thick absorbent paper (or lint-free cloth), with a soft towel placed underneath. (Bubbles that are not removed after patting dry can be punctured with a clean pipette tip).

Step 4: Color: Add 50μL of Substrate Reagent A to each well. Then add 50μL of Substrate Reagent B per well. Shake gently by hand (or use a microplate shaker) for 5s, and allow to react for 15min at 25°C in the dark. (The reaction can be extended appropriately if the blue color is too pale.)

Step 5: Stop the reaction: Pipette 50μL of Stop Solution to each well, and shake gently by hand (or use a microplate shaker). The reaction would be stopped.

Step 6: Calculate: Determine the Optical Density (OD value; absorbance value) at 450nm (Reference wavelength 630nm) with a microplate reader. Finish this step within 10min after stop the reaction.

7 Interpretation of result

7.1 Calculate the percentage of absorbance value

$$\text{Percentage of absorbance value}(\%) = \frac{A}{A0} \times 100\%$$

A—the average OD value of the sample or standard;

A0—the average OD value of the 0ppb standard.

It is used to calculate the percentage absorbance of a standard or sample.

7.2 Draw the standard curve and calculate

Take absorbance percentage (A/A0) of standards as Y-axis and the corresponding log of standards concentration (ppb) as X-axis.

Draw the standard semi-log curves with X-axis and Y-axis.

Take absorbance percentage of samples substitute into standard curve, then can get the corresponding concentration from standard curve. **Last, the resulting concentration values multiplied by the corresponding dilution times is the actual concentration of AFT of samples.**

If professional analysis software of the kit is used for calculation, it is more convenient for accurate and rapid analysis of a large number of samples.

8 Attention

8.1 Before test, the reagents and samples should be balanced to room temperature (25°C). If below 25°C, it will lead to all the standard OD value on the low side.

8.2 In the washing process, dry wells may result in non-linear standard curves and undesirable reproducibility. Therefore, proceed to the next step immediately after washing.

8.3 Please mix the contents within the wells uniformly and wash the plate thoroughly. The reproducibility is largely determined by consistency of washing step.

8.4 During the incubation, cover microplates with adhesive membrane to avoid light.

8.5 Do not use kits that are overdue. Do not mix reagents with those from other lots.

8.6 Substrate Reagent A/B is colorless. If not, please discard.

8.7 If absorbance value of 0ppb is below 0.5（A450nm< 0.5), it means that the reagent may be metamorphic.

8.8 Stop solution is corrosives, please avoid contact with skin.

8.9 As the OD values of the standard curve may vary according to the conditions of actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.

8.10 For the mentioned sample, fast and efficient extraction methods are included in the kit description. Please consult technical support for the applicability if other sample need to be tested.

8.11 The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS can be used for quantitative confirmation.

9 Storage conditions

The kit shall be stored at 2-8 °C. Avoid freezing.

Shelf Life: 12 months. The date of manufacture is presented in the label of the box.