Aflatoxin B1 (AFB1) ELISA Kit

Technical Manual

(ELISA)

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Shenzhen Finder Biotech Co.,Ltd.
Web: www.szfinder.com
Tel: +86 0755 23499025 Email: techsupport@szfinder.com
Add: Building B12,Life Science Industrial Park, KuiyongSubdistrict, Dapeng New Area, Shenzhen,China

1 Principle and Application

This kit adopts the method of indirect competitive enzyme-linked immunoassay (ELISA) to detect Aflatoxin B1 (AFB1) 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 AFB1 in the samples will compete with the coupled antigens to combine with anti-AFB1 antibodies. After adding HRP conjugate, take coloration with TMB substrates. Absorbance value of the samples is a negative correlation with AFB1 content. Lastly, by comparing the obtained absorbance values with the standard curve, we can calculate the content of AFB1 toxin in the sample.

2 Technique Data

2.1 Kit Sensitivity: 0.03ppb (ng/mL)

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

2.3 Detection Limits:

Sample	Detection Limits
Grains	0.15ppb
Samples with strong water absorption (such as corn, husk and bran)	0.6ppb
Edible oils such as peanut oil	0.6ppb
Food (such as sauces, cookies, pastries) or condiments	0.6ppb
Beer	0.3ppb
Wine, soy sauce, vinegar	0.15ppb
Tea leaf	0.2ppb
Triticeae crops (wheat and so on)	1.2ppb

2.4 Cross-reaction Rate:

Aflatoxin B1 ..... 100%

2.5 Sample Recovery Rate:

Sample	Recovery Rate
Grains	85±15%
Peanut oil	82±15%
Other edible oils	85±15%
Food (such as sauces, cookies, pastries) or condiments	83±15%
Beer	84±15%
Wine, soy sauce, vinegar	87±15%
Tea leaf, Triticeae crops (wheat and so on)	75±15%

3 Composition of the Kit

Reagent	Specification
Microtiter Plate	8wells× 12strips
Standard: 0ppb, 0.03ppb, 0.06ppb, 0.12ppb, 0.24ppb,0.48ppb (black cap)	1.0mL each
High Standard: 100ppb(black cap)	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), nitrogen evaporator, vortex mixer (for shake and mix), centrifuge, graduated transfer pipette, and balance with a division value of 0.01 g, constant temperature device;

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

4.3 Reagents: methanol, N-hexane, trichloromethane.

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).

Solution 3: Sample Redissolving Solution

35% Methanol solution(Methanol/Deionized water= 3.5: 6.5）

5.3 Sample pretreatment steps:

5.3.1 Grains 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: 0.15ppb

5.3.2 Samples with strong water absorption (such as corn husk and bran) treatment.

1) Weigh 2g±0.05g of homogenized samples into a 50mL centrifuge tube, pipette 20mL 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. (Samples with extremely high levels of toxins can be diluted with Sample Redissolving Solution (Solution 3) before testing. For example, take 0.1mL of the mixture in this step and add 0.9mL of Sample Redissolving Solution (Solution 3) to mix well. This is equivalent to the sample being diluted 200 times, and the detection limit is 6ppb.)

3) Take out 50μL for test.

Dilution times of the sample:20 Detection limits: 0.6ppb

5.3.3 Peanut oil and other edible oils treatment.

1) Take 2mL of samples into a 50mL centrifuge tube, pipette 8mL of N-hexane and 10 mL of Sample Extraction Solution (Solution 1), shake them for 5min and centrifuge them at 4000 rpm at room temperature for 10min.

2) Remove the upper liquid, take 0.5mL of the lower liquid, add 0.5mL of deionized water and mix them well. Then take 0.5mL of the mixed liquid into another tube, add 0.5mL of the Sample Redissolving Solution (Solution 3), and shake for 30 seconds.

3) Take out 50μL for test.

Dilution times of the sample:20 Detection limits: 0.6ppb

5.3.4 Food (sauces, cookies, pastries, etc.) or condiments treatment.

1) Weigh 1g±0.05g of homogenized samples a 15mL centrifuge tube, add 10mL of methanol, shake them for 5min and centrifuge at 4000 rpm at room temperature for 5min.

2) Take 2mL of supernatant into a 15mL centrifuge tube, dry it with nitrogen evaporator (using nitrogen or air) at 50°C-60°C.

3) Add 2mL of deionized water, shake for 30 seconds, then add 6mL of trichloromethane, shake for 5 minutes, and centrifuge at room temperature at 4000 rpm for 5 minutes.

4) Transfer 1.5mL of the lower layer liquid into a 10mL centrifuge tube, and dry it with nitrogen evaporator (using nitrogen or air) at 50°C-60°C.

5) Add 0.5mL of N-hexane, shake for 30 seconds, then add 1mL of Sample Redissolving Solution (Solution 3), shake for 1 minute, and centrifuge at room temperature at 4000 rpm for 5 minutes.

6) Remove the upper layer, take 50μL of the lower liquid for analysis.

Dilution times of the sample:20 Detection limits: 0.6ppb

5.3.5 Beer treatment.

1) Stir the beer thoroughly (to remove carbon dioxide), take 2mL of the sample, add 1mL of deionized water, then add 7mL of methanol. Shake them for 5 minutes.

2) Take 0.5mL of the well-mixed sample solution (obtained from the previous step) in a tube, then add 0.5mL of deionized water. Mix the solution thoroughly.

3) Take 50μL for analysis.

Dilution times of the sample:10 Detection limits: 0.3ppb

5.3.6 Wine, soy sauce, vinegar treatment.

1) Take 2mL of the sample, add 2mL of deionized water, then add 10mL of trichloromethane. Shake them for 5 minutes and centrifuge it at room temperature at 4000 rpm for 10 minutes.

2) Take 1mL of the lower layer liquid and dry it with nitrogen evaporator (using nitrogen or air) at 50°C -60°C.

3) Add 0.5mL of Sample Extraction Solution (Solution 1) to fully dissolve the dried material, then add 0.5mL of deionized water and mix thoroughly.

4) Take 50μL for analysis.

Dilution times of the sample:5 Detection limits: 0.15ppb

5.3.7 Tea leaf treatment.

1) Weigh approximately 2g ± 0.05g of the powdered sample into a 50mL centrifuge tube. Add 4mL of methanol, shake the mixture for 5 minutes, and centrifuge at room temperature at 4000 rpm for 10 minutes.

2) Take 0.3mL of the supernatant and add 0.6mL of deionized water. Mix thoroughly.

3) Take 50μL for analysis.

Dilution times of the sample:6 Detection limits:0.2ppb

5.3.8 Triticeae crops (wheat and so on) treatment.

1) Weigh 1g±0.05g of homogenized samples into a 50mL centrifuge tube, pipette 2mL of Sample

Extraction Solution (Solution 1), shake them for 5min and centrifuge at 4000 rpm at room temperature for 10min.

2) Take 0.2mL of supernatant, add 0.2mL of deionized water, shake for 30 seconds, and centrifuge at room temperature at 4000 rpm for 5 minutes.

3) Take 0.1mL of the centrifuged sample solution (Obtained from step 2)) and add 0.9mL of Sample Redissolving Solution (Solution 3). Mix the solution thoroughly.

4) Take 50μL of the mixture for analysis.

Dilution times of the sample:40 Detection limits: 1.2ppb

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 wells in sequence corresponding to the samples and standard, make 2-well parallel trials for each sample and standard, and record their locations.

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 in the dark.

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 tapping 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 AFB1 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.

8.8Stop 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.