

Aflatoxin B1 ELISA Kit

AfB1 ELISA

Product #: F1001-96T

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1. Description

Aflatoxin B1 is a derivate of dihydrofuran oxanaphthalene o-ketone, it is highly toxic to humans and animals, and its toxic effect is mainly liver injury. Aflatoxin B1 is the most common and most harmful toxin, thus strict maximum residue limit of aflatoxin B1 has been established in many countries and international organizations.

This ELISA Kit is based on indirect competitive ELISA to detect aflatoxin B1 in sample within 45 minutes.

2. Principle

This ELISA Kit is based on indirect competitive ELISA, the coupled antigen was pre-coated in the microwells, the aflatoxin B1 will compete the antibody with the pre-coated antigen, then add enzyme conjugate and TMB substrate successively, the OD value of the sample is negatively correlated with the content of aflatoxin B1 contained in the sample. The standard curve is fitted by regression and multiplied by the corresponding dilution ratio to obtain the content of aflatoxin B1 in the sample.

3. Application

This kit is applicable for quantitative determination of aflatoxin B1 in feed, peanut, cereal, nut, sunflower seed oil and linseed oil.

4. Instrument and material required

- 1) ELISA reader, with 450/630nm
- 2) Centrifuge
- 3) incubator
- 4) Balance, 0.0001g
- 5) Centrifuge tube, 2mL, 15mL, 50mL
- 6) Vortex mixer
- 7) Micropipette, 20-200 μ L, 100-1000 μ L
- 8) Multi-channel pipette, 300 μ L

5. Reagent required

Ultrapure water, Methanol, Acetonitrile

6. Kit components

- 1) Microtiter plate, 96wells, 1 plate
- 2) Aflatoxin B1 standards, 1mL/vial, 6 vials, 0, 0.05, 0.10, 0.20, 0.40, 0.60 ng/mL
- 3) Enzyme conjugate, 7mL, with brown cap
- 4) TMB Substrate, 12mL, with brown cap
- 5) 20X Wash buffer, 50mL, with transparent cap
- 6) 2X Sample buffer, 50mL, with transparent cap
- 7) manual, 1 set

7. Buffer preparation

Wash buffer: Dilute 20X wash buffer with ultrapure water, in the volume ratio of 1:19, for example, 10mL 20X wash buffer + 190mL ultrapure water, mix thoroughly. This diluted wash buffer can be stored at 2-8°C for 1 month.

Sample buffer A: Dilute 2X sample buffer with ultrapure water, in the volume ratio of 1:1, for example, 10mL 2X sample buffer + 10mL ultrapure water, mix thoroughly. This diluted wash buffer can be stored at 2-8°C for 1 month.

Sample buffer B: Dilute 2X sample buffer with ultrapure water, in the volume ratio of 2:1, for

example, 20mL 2X sample buffer + 10mL ultrapure water, mix thoroughly. This diluted wash buffer can be stored at 2-8°C for 1 month.

50% methanol: Dilute methanol with ultrapure water, in the volume ratio of 1:1, for example, 10mL methanol + 10mL ultrapure water, mix thoroughly, which can be used in the extraction of sample.

80% acetonitrile: Dilute acetonitrile with ultrapure water, in the volume ratio of 4:1, for example, 40mL acetonitrile + 10mL ultrapure water, mix thoroughly, which can be used in the extraction of sunflower seed oil and linseed oil sample.

Stop solution: 2M sulfuric acid, which should be prepared by customer themselves.

8. Notice and precautions before assay

- 1) Make sure the ELISA kit and all reagents are returned to room temperature (20-25 °C). For example, keep these reagent and kits at room temperature for at least 30min.
- 2) Return unused kit components to 2-8 °C.
- 3) Washing step is important for the reproducibility of the kit, please follow this instruction carefully.
- 4) Cover the ELISA plate during all incubation. Avoid direct sunlight.

9. Sample preparation

Precautions before prepare samples:

- 1) Use disposable tips during the test. Change new tip for different sample / reagent.
- 2) Make sure all lab wares are clean and ready to use.
- 3) Prepared sample shall be analyzed immediately after dilution.

10. Sample handling

10.1 Feed

Take 1 ± 0.05 g sample into a 15mL centrifuge tube, then add 5mL of 50% methanol, vortex for 2min, and water bath for 10min at 50°C. Centrifuge for 5min at 5000g, and then take 200µL supernate into a 2mL centrifuge tube, add 600µL sample buffer A, mix thoroughly. Take 50µL sample for assay.

10.2 Peanut, cereal

Take 1 ± 0.05 g sample into a 15mL centrifuge tube, then add 3mL of 50% methanol, vortex for 2min. Centrifuge for 5min at 5000g, and then take 100µL supernate into a 2mL centrifuge tube, add 700µL sample buffer A, mix thoroughly. Take 50µL sample for assay.

10.3 Nut (except for peanut)

Take 1 ± 0.05 g sample into a 15mL centrifuge tube, then add 7mL of 50% methanol, vortex for 2min. Centrifuge for 5min at 5000g, and then take 200µL supernate into a 2mL centrifuge tube, add 800µL sample buffer B, mix thoroughly. Take 50µL sample for assay.

10.4 Sunflower seed oil, linseed oil

Take 1mL sample into a 50mL centrifuge tube, then add 19mL of 80% acetonitrile, vortex for 2min. Centrifuge for 5min at 5000g, and then take 100µL supernate into a 2mL centrifuge tube, add 900µL sample buffer B, mix thoroughly. Take 50µL sample for assay.

11. Assay procedures

- 1) Return the ELISA kit and all reagents to room temperature (20-25 °C). For example, keep these reagent and kits at room temperature for at least 30min.
- 2) Take needed microwells and zip rest in the zip-bag and return to 2-8 °C.
- 3) Layout the plate and record sample and standard well positions. It is recommended to run

all tests in duplicates.

- 4) **Add sample/standard, enzyme conjugate:** add sample/standard into the wells, 50µL per each, then add enzyme conjugate, 50µL per well, shake gently and then cover the plate and incubate at **25 °C for 30min**.
- 5) **Wash:** take out the plate and pour the liquid out. Use the diluted wash buffer to wash the plate, 250µL per well. Wash for 4-5 times with interval of 10s. The pour the liquid out and tap the plate against absorbent paper. Eliminate the air bubble in the wells with micropipette tip if the bubble exists.
- 6) **Coloration:** add TMB substrate, 100µL per well, and then cover the plate and incubate **25 °C for 15min**.
- 7) **Stop the reaction:** add stop solution, 50µL per well, shake gently and read the plate with ELISA reader at 450nm. Read the plate within 5min after adding stop solution.

12. Result Calculation

This kit is based on competitive ELISA, thus the OD values is inversely proportional to the aflatoxin B1 content contained in sample.

12.1 Quantitative analysis

Calculation of absorbance: the percentage absorbance of standard product or sample is equal to the average absorbance value of standard product or sample (double hole) divided by the average absorbance value of blank standard product, and then multiplied by 100%, i.e

$$\text{percentage absorbance (\%)} = \frac{B}{B_0} \times 100\%$$

B - the average absorbance value of standard product or sample

B₀- the average absorbance value of blank standard product

12.2 Standard curve

The standard curve was drawn by taking the percentage absorption of standard substance as the vertical coordinate and the concentration of standard substance aflatoxin B1 as the horizontal coordinate. Put the percentage absorption rate of samples into the standard curve, read the corresponding concentration of samples from the standard curve, and multiply the corresponding dilution times to obtain the actual concentration of aflatoxin B1 in samples.

13. Sample dilution factor:

Feed: 24 times

Peanut, cereal: 32 times

Nut (except for peanut): 40 times

Sunflower seed oil, linseed oil: 200 times

14. LOD of sample

Feed: 1.2 ng/g

Peanut, cereal: 1.6 ng/g

Nut (except for peanut): 2 ng/g

Sunflower seed oil, linseed oil: 10 ng/mL

15. Specifications of the kit

- 1) Sensitivity: 0.05ng/mL (ppb)
- 2) Specificity: 100% to aflatoxin B1
- 3) Recovery: 80% - 110%
- 4) Precision: CV<10%.

16. Cautions and tips for the test

- 1) Lower room temperature, e.g., lower than 20 °C may cause lower OD values. Please make sure all reagent and kit components are returned to room temperature.
- 2) Wash step is vital for the reproducibility of the kit. Please wash according to the kit instruction. Do not let the plate dry during wash. Continue the next operations immediately after wash step.
- 3) Shake each reagent gently before use.
- 4) Stop solution is acidic, please handle with care.
- 5) Do not use expired kits and reagents. Do not mix the reagent and kits from different LOT.
- 6) The kit is stored at 2-8°C(36-46°F), do not freeze.
- 7) TMB substrate is sensitive to sunlight. Avoid direct sunlight.
- 8) If Standard 1 (0ng/L) OD is lower than 0.5, please do not use. The kit may be expired or deteriorated.
- 9) The coloration step takes 15min. You can prolong it to 20min-25min if the color of the well is too light. On the contrary, please reduce the incubation time.
- 10) The incubation is 25°C /98.6 °F, lower or higher temperature will cause changes of OD and sensitivity of the kits, which may affect the result of the assay.

17. Storage and expiration

The kit is valid for 12months when stored at 2-8 °C. LOT and Expiry information are printed on the package.