Aflatoxin M1 ELISA Kit

AfM1 ELISA

Product #: E6002-96T

V733.04

Manufacturer Information

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1. Description

Aflatoxin M1 is a metabolite of aflatoxins, the most carcinogenic and dangerous natural biotoxins. Due to the contamination of feed and feed stuff, cow milk can easily be contaminated by aflatoxins, thus strict maximum residue limit of aflatoxin M1 has been established in many countries and international organizations.

This ELISA Kit is based on indirect competitive ELISA to detect aflatoxin M1 in milk and milk powder.

2. Application

This kit is applicable for determination of aflatoxin M1 in milk, milk powder, fermented milk。

3. Kit components

1) Microtiter plate, 96wells, 1 plate
2) Aflatoxin M1 standards, 1mL/vial, 6 vials, 0, 5, 10, 25, 50, 100 ng/L
3) Enzyme conjugate, 12mL, with brown cap
4) Antibody solution, 7mL, with green cap
5) TMB substrate, 12mL, with brown cap
6) Stop solution 7mL, with white cap
7) 20X Wash buffer, 50mL, with transparent cap

4. Instrument and material required

1) ELISA reader, with 450/630nm
2) Water bath
3) Centrifuge
4) Incubator
5) Balance, 0.0001g
6) Centrifuge tube, 2mL, 15mL
7) Vortex mixer
8) Micropipette, 5-50μL, 20-200μL, 100-1000μL
9) Multi-channel pipette, 300μL
10) graduated pipette,10mL

5. Reagent required

Ultrapure water, Absolute ethyl alcohol, Acetonitrile, n-hexane

6. Buffer preparation

Buffer 1 Wash buffer: Dilute 20X wash buffer with ultrapure water, in the volume ratio of 1:19, fox example, 10mL 20Xwash buffer + 190mL ultrapure water, mix thoroughly.

This diluted wash buffer can be stored at 2-8°C for 1 month.

Buffer 2 Sample buffer: Dilute 4X sample buffer with ultrapure water, in the volume ratio of 1:3, for example, 10mL 2X sample buffer + 30mL ultrapure water, mix thoroughly.

This diluted sample buffer can be stored at 2-8°C for 1 month.

Buffer 3 Skimmed milk solution: Weight 1.0000g±0.0100g skimmed milk powder, then add 10ml ultrapure water to dissolve it.

7. Sample preparation

7.1 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.

7.2 Milk

Take 1mL milk sample into a 2mL centrifuge tube, then take 50μL for assay.

7.3 Milk powder

1) Take 1.0±0.01g sample into a new 15mL centrifuge tube, add 8mL ultrapure water, vortex for 1min to dissolve.
2) Take 1mL dissolved sample into another 2mL tube, then take 50μL for assay.

7.4 Fermented milk

1) Take 2.0±0.01g sample into a 15mL centrifuge tube, add 0.3mL absolute ethyl alcohol, then add 1.7mL skimmed milk solution, vortex for 1min to dissolve.
2) Centrifuge for 10min at 4000g, and then take 50μL supernate liquid for assay.

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 60min.
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. 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 60min.
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, antibody**: add sample/standard into the wells, 50μL per each, then add antibody solution, 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 (buffer 1) 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) **Add enzyme conjugate**: add enzyme conjugate, 100μL per well, shake gently and then incubate at **25°C for 30min**. Then take out and repeat **Wash Step**.
7) **Coloration**: add TMB substrate, 100μL per well, and then cover the plate and incubate **25 °C for 15min**.
8) **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.

10. Result Calculation

This kit is based on competitive ELISA, thus the OD values is inversely proportional to the NPE content contained in sample. With ELISA reader, a standard curve can be plotted with the ODs obtained. You can use the NPE standards to finish the standard curve, horizontal axis is the logarithm of the concentration of NPE standards, vertical axis is the OD percentage of standards. Please note that the sample concentration you get from the standard curve is the diluted sample.

$$\text{OD Percentage } (\%) = \frac{B}{B0}$$

B – mean OD of standards or samples
B0 – mean OD of first standard (0 ng/L)

11. Specifications of the kit

1) Sensitivity: 5ng/L
2) Specificity: 100% to aflatoxin M1
3) Dilution factor:
   milk-1; milk powder-1; fermented milk-2
4) LOD (Detection limit):
   milk-5ng/L; milk powder-5ng/kg; fermented milk-10ng/kg.

12. 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, lower or higher temperature will cause changes of OD and sensitivity of the kits, which may affect the result of the assay.

13. Storage and expiration

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