AFLATOXIN M1

Cat # GEB24007

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Background

Aflatoxin M1 is a hydroxylated metabolite of aflatoxin B1 that is excreted in milk in both humans and lactating animals. Approximately 0.3% to 6.2% of the ingested aflatoxin B1 is converted to aflatoxin M1 in the liver of lactating animals. Even though it is less toxic than aflatoxin B1, aflatoxin M1 has hepatotoxic and carcinogenic effects. International Agency for Research on Cancer (IARC) has placed aflatoxin M1 with aflatoxin B1 as a Group 1 carcinogen [1]. Many studies have shown that the presence of aflatoxin M1 in milk and milk products is a health issue because these products are regularly consumed in the daily diet [2]. While the maximum residue limit (MRL) of aflatoxin M1 in milk is 500 ng/kg in the United States, Brazil and most Asian and African countries, it is 50 ng/kg in most European countries.

Intended use

The kit is designed for the quantitative detection of aflatoxin M1 over a wide range (5 – 500 ng/L, ppt). For research use only – not intended for diagnostic use.

Assay principle

Aflatoxin M1 kit is based on competitive enzyme-linked immunosorbent assay technology. Aflatoxin in either the sample or standard binds to an antibody specific to aflatoxin that is coated on the plate. After incubation, the wells are rinsed to remove any unbound aflatoxin. An aflatoxin-HRP conjugate is then added, which binds to the antibody sites not occupied by aflatoxin. After a second incubation, the wells are rinsed again to remove any unbound aflatoxin-HRP conjugate. The bound HRP conjugate then develops blue color after the addition of TMB substrate. The intensity of the blue color developed is inversely proportional to the concentration of aflatoxin in the sample and standard. The color development will stop by addition of Acid stop solution, and the blue color turns to yellow. The intensity (optical density; OD) of yellow color is measured at a 450 nm. The concentration of aflatoxin in the sample is calculated on the basis of a standard curve.

Materials provided

Component	Amount
Antibody coated plate (96 wells, clear)	1 pouch
Standards at 0, 5, 15, 50, 150 and 500 ng/L	3 mL x 12 vials
Aflatoxin-HRP conjugate	10 mL x 2 bottles
Milk diluent	12 mL x 1 bottle
TMB substrate	10 mL x 2 bottles
Acid stop	12 mL x 1 bottle
PBST wash buffer	1 pouch

Materials required but not provided

- Microplate reader (450 nm filter)
- Microcentrifuge (≥ 3000 rpm)
- Microcentrifuge tube
- Single-channel pipette: 20 - 200 µL, 100 µL – 1000 µL
- Multi-channel pipette (8-channel): 20 – 200 µL
- Vortex mixer
- Analytical balance
- Graduated cylinder
- Distilled or deionized water
- Wash bottle
- Timer
- Paper towel

Precautions

- Wear protective gloves, lab coat, eye, and face protection.
- Store the kit at 2-8°C at all times.
- Do not mix the reagents from different lots.
- TMB Substrate must be colorless and protected from light.
- The Acid Stop is a strong acidic solution. If exposed, flush with water and immediately see a doctor.
- Dispose all materials in a waste container.
- The waste container must be treated with 5 - 6% sodium hypochlorite or commercial bleach, and allow at least 30 minutes before discard.

Sample procedure

Milk (Liquid)

1. Centrifuge cold milk at 3000 x g for 10 minutes.
2. Remove the upper fat layer.
3. Use the defatted milk for the assay once it reaches room temperature.

Milk (Powder)

1. Weigh 10 g milk powder and fill up to 100 mL with distilled or deionized water.
2. Dissolve completely by shaking or stirring.
3. Take a small portion and cool it in the refrigerator.
4. Centrifuge at 3000 x g for 10 minutes.
5. Remove the upper fat layer.
6. Use the defatted milk for the assay once it reaches room temperature.

Assay procedure

1. Bring all the components to room temperature (20 – 25 °C) before use.
2. Reconstitute PBST wash buffer by adding the content into a container with 1-Liter of water.
3. Determine the number of antibody coated wells to be used for samples and standards.
4. Dispense **200 µL** of standard or sample extract into each well.
5. Cover the plate and incubate for **15 minutes** (1st incubation).
6. Decant the contents of the wells into an appropriate waste container. Invert the plate and tap on paper towel.
7. Dispense **200 µL** of standard or sample extract into each well.
8. Cover the plate and incubate for **15 minutes** (2nd incubation).
9. Decant the content and rinse with PBST wash buffer by filling and decanting 3 times.
10. Invert the plate and tap on paper towel to remove residual wash buffer.
11. Dispense **150 µL** of Aflatoxin-HRP conjugate. Cover the plate and incubate for **15 minutes**.
12. Decant the content and rinse with PBST wash buffer by filling and decanting 5 times.
13. Invert the plate and tap on paper towel to remove residual wash buffer.
14. Dispense **150 µL** of TMB substrate and incubate for **15 minutes**. Cover the plate to avoid direct exposure to light.
15. Dispense **100 µL** of Acid stop to each well in the same pattern as TMB substrate.
16. Read absorbance (optical density, OD) at 450 nm.
17. Calculate % binding for each standard and sample dividing its OD value (B) by zero standard OD value (B_0) and multiplying by 100 to express as a percentage (% B/B_0).

Standard curve and calculation

Construct a standard curve by plotting the mean % B/B0 values of standards on Y-axis and corresponding concentrations of aflatoxin M1 on X-axis using a 4-parameter logistic fit. Calculate aflatoxin M1 concentration of sample by applying % B/B_0 value. For the powdered milk sample, multiply the calculated concentration by **10** to take into account dilution factor in order to express the determined concentration as ng/kg. If a sample contains aflatoxin M1 at a greater than 500 ng/L, the sample should be diluted further with the milk diluent provided.

Cross-reactivity

Aflatoxin	Cross reactivity
M1	100%
B1	< 7%
B2	< 0.01%
G1	< 0.01%
G2	< 0.01%

References

1. IARC. (2002). IARC monographs on the evaluation of carcinogenic risks to humans. In Traditional herbal medicines, some mycotoxins, mapthalene and styrene (Vol. 82) Lyon: IARC Press.

2. Ketney, O.; Santini, A.; Oancea, S. Recent aflatoxin survey data in milk and milk products: A review. *Int. J. Dairy Technol.* **2017**, *70*, 320–331.