Aflatoxin M_1 ELISA Kit

Competitive enzyme immunoassay kit for quantitative analysis of Aflatoxin M_1

Catalog Number: EL2053-01

For Research Use Only. Not for use in Diagnostic Procedures.

1. Background

Aflatoxins are secondary metabolites produced by a variety of strains of fungi, like *Aspergillus flavus* and *A. parasiticus*. Aflatoxin M_1 (AFM1) is the hydroxylated metabolite of aflatoxin B_1. AFM1 can be found in milk produced from livestock that have consumed contaminated feed. The high toxicity and carcinogenic properties of Aflatoxins are well known and pose a major threat to human health.

2. Test Principle

The Aflatoxin M_1 plate kit is a competitive enzyme-labeled immunoassay. The Aflatoxin M_1 sample extract and calibrators are pipetted into the test wells followed by the Aflatoxin M_1 antibody into the test wells to initiate the reaction. During the 30 minutes incubation period, Aflatoxin M_1 from the sample and Aflatoxin M_1 antigen compete for binding to the Aflatoxin M_1 antibody. The Aflatoxin M_1 antibody is captured on the walls of the test well. Following this 30-minute incubation, the contents of the wells are removed and the wells are washed to remove any unbound Aflatoxin M_1 and free Aflatoxin M_1 antibody. After wash, 1X HRP-conjugated Antibody#2 is added for 30 minutes incubation. The wells are washed afterwards, and a clear substrate is then added to the wells and any bound enzyme conjugate causes the conversion to a blue color. Following a 15-minute incubation, the reaction is stopped and the amount of color in each well is read. The color of the unknown samples is compared to the color of the calibrators and the Aflatoxin M_1 concentration of the samples is derived.

3. Applications

This kit can be used for quantitative analysis of Aflatoxin M_1 in samples such as raw milk and powdered milk.

4. Equipment and Reagents Needed (not provided)

4.1 Equipment

- ELISA Reader (450nm/630nm)
- Deionized water
- Vortex mixer
- Centrifuge
- Timer
- Wash bottle
- Polystyrene centrifuge tube: 50mL, 2mL
- Micropipettes: 20μL-200 μL, 100μL-1000μL, tips
- Multi-channel pipette: 8-channel (50, 100 & 150 μL)

5. Components Provided in This Kit

- Microtiter plate with 96 wells coated with Aflatoxin M_1
- Aflatoxin M_1 Standards (6 vials × 0.8mL/vial): 0ppb (green cap), 0.005ppb (purple cap), 0.015ppb (yellow cap), 0.03ppb (blue cap), 0.09ppb (orange cap), 0.3ppb (red cap)
- Aflatoxin M_1 Antibody#1: 11mL
- 100X HRP-Conjugated Antibody#2: 0.25mL
- Antibody#2 Diluent: 20mL
- 20X Wash Solution: 28mL
- TMB Substrate Solution: 12mL
- Stop Solution: 14mL

6. Reagents Preparation

- 1X Wash solution: combine one volume of the 20X Wash Solution with 19 volumes of deionized water. Mix well.
- 1X HRP-conjugated Antibody#2: combine one volume of the 100X HRP-Conjugated Antibody#2 with 99 volumes of Antibody#2 Diluent. Vortex for 10 seconds to mix.
  ☛ Prepare this solution fresh before each test.

7. Notice and Precautions Before Operation

- Please use a fresh tip in the process of experiment and change the tips when absorbing different reagent.
- If running more than two strips at once, the use of a multichannel pipette is required.
- Make sure that all experimental instruments are clean.
- Treated samples can be stored at 2-8°C for 24 hours in the dark.

8. Sample Preparation

8.1 Raw milk

The milk must be tested within 24 hours after milking. Refrigerate the sample at 2-8°C.

- Raw milk can be tested directly in the assay.
- If necessary, centrifuge it at 4000g for 10 minutes.
- Separate the upper fat layer from the skim milk.
- Take 50μL of the skim milk for assay.

*For samples higher than 0.3ppb, dilute the samples with deionized water. Multiply the results by the dilution factor used.

8.2 Powdered milk

- Weigh 1 g of powdered milk, add 10mL of deionized water. Vortex to dissolve.
- If necessary, centrifuge it for 10 minutes.
- Separate the upper fat layer from the skim milk.

- Take 50µL of the skim milk for assay.
- Dilution factor is 10.

9. Assay Process

9.1 Instructions Prior to Beginning Assay

1. Ensure that all reagents and microwells are at room temperature (20-25°C).
2. Return all reagents to 2-8°C immediately after their use.
3. Wash the microwells correctly; this is a vital factor in the reproducibility of the ELISA analysis.
4. Avoid direct sunlight during the incubation.

9.2 Steps in the Assay Process

1. Take all reagents out at room temperature (20-25°C) for more than 30 minutes. Shake gently before use.
2. Take out the needed microwells and return the rest into the zip-lock bag at 2-8°C immediately.
3. The diluted wash solution should be brought to room temperature before use.
4. Number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.
5. Dispense **50µL of Aflatoxin M_1 Standards or sample** into each well.
6. Dispense **100µL of Antibody#1** into appropriate test wells.
7. Shake the plate gently for 30 seconds using a back-and-forth motion.
8. Cover the plate. Incubate for **30 minutes** at room temperature.
9. Decant the contents of the wells into an appropriate waste container.
10. Rinse the microwells with 250µL of the 1X Wash Solution 3 times.
11. Absorb the residual solution by inverting with absorbent paper to remove the last of the Wash Solution.
12. Add **150µL of 1X HRP-Conjugated Antibody#2** (freshly prepared) to each well.
13. Shake the plate gently for 30 seconds using a back-and-forth motion.
14. Cover the plate. Incubate for **30 minutes** at room temperature.
15. Decant the contents of the wells into an appropriate waste container.
16. Rinse the microwells with 250µL of the 1X Wash Solution 3 times.
17. Absorb the residual solution by inverting with absorbent paper to remove the last of the Wash Solution.
18. Add **100µL TMB Substrate Solution** to each well, mix by shaking the plate gently and incubate for **15 minutes** at 25°C with cover.
19. Add **100µL of Stop Solution** to each well. Mix gently by shaking the plate manually and measure the absorbance at 450nm (Read the result within 5 minutes after addition of Stop Solution).

10. Results

10.1 Calculating the Percentage absorbance

- The mean values of the absorbance values obtained from the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%.

Absorbance (%) = B / B_0 *100

B = the mean absorbance value of each standard or each sample

B_0 = absorbance value of zero standard

10.2 Drawing a Standard Curve

- To draw a standard curve, the absorbance value of standards as y-axis, semilogarithmic of the concentration of the standards (ppb) as x-axis.
- The concentration of each sample (ppb), which can be read from the standard curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.
- Sample dilution factor: If the absorbance of a sample is lower than the highest calibrator (0.3 ppb), the concentration of Aflatoxin M_1 is too high and out of range of the standard curve. Dilute the sample and rerun. Samples should be diluted to fit into the standard curve (0.005ppb to 0.3ppb). Results must then be multiplied by the dilution factor used.

11. Sensitivity, Accuracy and Precision

11.1 Detection limit:

- Raw milk 0.025ppb
- Powdered milk 0.08ppb

11.2 Accuracy:

- Raw milk, powdered milk ..... 80±10%

11.3 Precision:

- C.V. of the ELISA kit .....less than 10%

12. General Instructions

12.1 Temperature of Reagents and Samples

- The mean values of the absorbance values obtained for the standards and the samples will be reduced if the reagents and samples have not been restored to room temperature (20-25°C).

12.2 Microwells

- Do not allow Microwells to dry between steps to avoid unsuccessful reproducibility and operate the next step immediately after tapping the microwells holder.

12.3. Shaking of Reagents

- Shake each reagent gently before use.

12.4. Skin Protection

- The Stop Solution is 0.75N HCl, keep your skin away from it.

12.5 Out of Date Kits

- Don't use kits that are expired. Don't exchange the reagents of different batches, or else it will drop the sensitivity.

12.6 General Comments

- Keep the ELISA kits at 2-8°C, do not freeze. Store the unused microwell plates back to the foil pouch. Avoid straight sunlight during all incubations. Covering the microtiter plate is recommended.

12.7 Special Issues Concerning Solutions and Reagents

- Substrate solution should be abandoned if it turns color. The reagents may turn bad if the absorbance value (450/630nm) of the zero standard is less than 0.5 (A450nm<0.5).

12.8 Special Issues Concerning Color

- The coloration reaction takes 15 minutes after the addition of TMB Substrate, but you can prolong the incubation time ranges to 35 minutes or more if the color is too light to be determined, never exceed 40 minutes, on the contrary, shorten the incubation time properly.

12.9 Incubation Temperatures

- Incubation temperature should be at room temperature (20-25°C). Higher or lower temperature on the day of testing will lead to experiment-to-experiment changes.

13. Storage

- Storage condition: 2-8°C
- Storage period: 12 months

Customer Notes:

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