AFM1 ELISA Kit

Cat#: orb411504 (ELISA Manual)

INTRODUCTION

Aflatoxin M1 is one of Aflatoxin with similar structure compounds. On damp-heat areas, the occurrence of Aflatoxin is highest in food and feed. Aflatoxin M1 with quite stable physical and chemistry properties, is not destroyed by pasteurization. When the mammal animal ingest food or feed containing Aflatoxin B1, it can be converted to the Aflatoxin M1 by hydroxylation. The main harm of Aflatoxin M1 is carcinogenicity and mutagenicity, destroying human and animal liver tissue, resulting in hepatocarcinoma and even death.

This kit is a detection product developed based on ELISA technology, with operation time as short as 50 min and a sensitivity of 0.02 ppb, and linear range from 0.02 ppb to 1.62 ppb.

PRINCIPLE OF THE ASSAY

This assay employs the competitive inhibition enzyme immunoassay technique.

The microtiter plate provided in this kit has been pre-coated with Aflatoxin M1 antigen. Standards or samples are added to the appropriate microtiter plate wells with Aflatoxin M1 specific antibody and Horseradish Peroxidase (HRP) conjugated anti-antibody. The competitive inhibition reaction is launched between pre-coated Aflatoxin M1 and Aflatoxin M1 in standards or samples with the Aflatoxin M1 special antibody. A substrate solution is added to the wells and the color develops in opposite to the amount of Aflatoxin M1 in the standards or samples. The color development is stopped and the intensity of the color is measured.

DETECTION RANGE

0.02 ppb-1.62ppb

SENSITIVITY

The minimum detectable dose of the kit is typically less than 0.02ppb.

The sensitivity of this assay, or Lower Limit of Detection (LOD) was defined as the lowest concentration that could be differentiated from zero. It was determined the mean OD value of 20 replicates of the zero standard added by their three standard deviations.

CROSS-REACTION RATE

Aflatoxin M1	100%
Aflatoxin B1	30%

RECOVERY RATE

Milk	70%-120%
Milk Power	70%-110%

LIMIT OF DETECTION

Milk	0.1 ppb
Milk Power	0.5 ppb

PRECISION

Intra-assay Precision (Precision within an assay): CV%<10%

Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): CV%<10%

Three samples of known concentration were tested in twenty assays to assess.

LIMITATIONS OF THE PROCEDURE

- The kit should not be used beyond the expiration date on the kit label.

- Do not mix or substitute reagents with those from other lots or sources.

- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

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MATERIALS PROVIDED

Reagent	Quantity(96T)	Quantity(48T)
Assay plate	96T	48T
Standard	6 x 1mL	6x 0.5mL
HRP-conjugate	1 x7mL	1 x 3.5mL
Antibody	1 x7 mL	1 x 3.5mL
TMB Substrate	1 x 12 mL	1 x 6 mL
Stop Solution	1 x10mL	1 x 5mL
Wash Buffer(10×)	1 x 50 mL	1 x 25mL
Adhesive Strip	4	4
Instruction Manual	1	1

STANDARD CONCENTRATION

Standard	S0	S1	S2	S3	S4	S5
Concentration (ppb)	0	0.02	0.06	0.18	0.54	1.62

STORAGE

Unopened kit	Store at 2 -8°C. Do not use the kit beyond the expiration date
Opened kit	May be stored for up to 1 month at 2 -8° C.

*Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- An incubator which can provide stable incubation conditions up to 25°C
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Centrifuge, Vortex mixer
- Analytical balance,2 decimal place
- Absorbent paper for blotting the microtiter plate.
- Single-channel micropipette(20μL-200μL、100μL-1000μL)

- 300μL multichannel micropipette

- 100 mL and 500 mL graduated cylinders.

- Deionized or distilled water.

- Pipettes and pipette tips.

- Test tubes for dilution.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

Note:

- Kindly use graduated containers to prepare the reagent.

- Bring all reagents to room temperature (20-25°C) before use for 30 min.

- Only the disposable tips can be used for the experiments and the tips must be changed when used for different reagents.

- Distilled water is recommended to be used to make the preparation for reagents or samples. Contaminated water or container for reagent preparation will influence the detection result.

REAGENT PREPARATION

- **Wash Buffer (1x)**: If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 10 mL of **Wash Buffer (10x)** into 90 mL deionized or distilled water to prepare 100 mL of **Wash Buffer (1x).** Keep it at 4℃ for one month.

Note:

1. Biorbyt is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.

2. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

3. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

SAMPLE COLLECTION AND STORAGE

A. Milk

1. Bring the milk sample to room temperature.

2. Take 50 µL of sample for further analysis.

Dilution factor of the samples: 1

B. Milk power

1. Weigh 1.00±0.05g of the milk power. Add 4 mL of deionize water, shake well.

2. Take 200 µL of sample and add 200 µL of deionize water, shake well.

3. Take 50 µL of sample for further analysis.

Dilution factor of the samples: 8

STORAGE

The prepared sample maybe stored for up to three days at 2-8°C.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature (20~25°C) before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.

1.   Prepare all reagents and samples as directed in the previous sections.

2.   Determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 2-8°C.

3.   Add 50 µL of **Standard** or **Sample** per well. Then add 50 µL of **HRP-conjugate** to each well and 50 µL of **Antibody** to each well. Cover the microtiter plate with a new adhesive strip and mix well, then incubate for 30 min at 25°C.

4.   Aspirate each well and wash, repeating the process four times. Wash by filling each well with 250 µL of **Wash Buffer (1x)** using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 30 seconds, complete removal of liquid at each step is essential to good performance.

5.   Add 100 µL of **TMB Substrate** to each well, mix well. Incubate for 15 minutes at 25°C. Protect from light.

6.   Add 50 µL of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.

7.   Determine the optical density of each well within 5 min, using a microplate reader set to 450 nm (Recommend to read the OD value at the dual-wavelength: 450/630 nm within 5 min).

Note:

1.   The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.

2.   Samples or reagents addition: Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 min. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

3.   Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.

4.   Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.

5.   Controlling of reaction time: Observe the change of color after adding Substrates (e.g. observation once every 10 min). Substrates should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.

6.   Substrates are easily contaminated. Substrates should remain colorless or light blue until added to the plate. Please protect it from light.

7.   Stop Solution should be added to the plate in the same order as the Substrates. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrates.

CALCULATION OF RESULTS

There are two methods to judge the results: the first one (A) is the rough judgment, while the second (B) is the quantitative determination. Note that the OD value of the sample has a negative correlation with Aflatoxin M1in the sample.

A:

Compare the sample average absorbance values with standards values, the Aflatoxin M1 concentration in the samples can be concluded. For example, the absorbance value of sample 1 is 0.799, the absorbance value of sample 2 is 1.129; absorbance values of standard are:1.926,1.779,1.548, 0.911,0.289, 0.194 and the corresponding concentrations are:0 ppb, 0.02 ppb, 0.06 ppb, 0.18 ppb, 0.54 ppb, 1.62 ppb; then the Aflatoxin M1in sample 1 and sample 2 are 0.18 ppb-0.54 ppb and 0.06 ppb-0.18 ppb; Lastly the reader is multiplied by the corresponding dilution factor of each sample and the actual concentration of sample is obtained

B:

The software offered together will facilitate the calculation process, it's suitable for accurate and fast analysis of large scale samples, please contact us.

Note:

• Discard the substrate with any color that indicates the degeneration of this solution; when the absorbance value of standard solution 0 of less than 0.5 indicates its degeneration.

• The optimum reaction temperature is 25℃, and too high or too low will result in the changes in the absorbance value and detecting sensitivity.