Aflatoxin M1 Plate Kit

Catalogue Number. orb59529

General description

The ELISASreening Kit is a competitive enzyme-labeled immunoassay for the quantitation of Aflatoxin M1 (AFM1) in milk and milk products. The test principle is based on a direct competitive enzyme-linked Immunosorbent assay (dcELISA). In the assay, AFM1 in the milk sample with afaltoxin-HRP enzyme conjugates for a limited amount of antibody which has been coated on the bottom of the test wells.

Materials and reagents supplied

1. One microtiter plate containing 6 strips of 16 test wells coated with polyclonal antibody specific to Aflatoxins
2. One vial of 【Negative control】, 1.5 mL
3. One vial each of 0.01, 0.05, 0.1, 0.25, and 0.5 ppb Aflatoxin M1 standard (calibrator), 1.5 mL
3. One vial of【HRP conjugate】, 6 mL
4. One vial of 【Substrate】, 12 mL
5. One vial of 【Stop solution】, 12 mL
6. One vial of 【10 x Wash buffer】, 30 mL

Additional materials not provided

1. 10 ml syringes
2. 0.45 micron, non-sterile filter units
3. Pipette and centrifuge tubes
4. Marking pen
5. Tape or Parafilm
6. Distilled water or tap
7. Microtiter plate reader or strips reader
8. Absorbent paper towels
9. Timer
10. Extraction buffer: MeOH, ACS grade
11. Calculator (Optional)
12. A multi-channel pipette (Optional)
13. Orbital shaker (Optional)
14. Solid phase extraction devices (Optional)
15. Microtiter plate washer (Optional)

Sample preparation

1. Pipette 1 mL of milk into a microcentrifuge tube, centrifuge at 13,000 rpm for 5 minutes (Milk powder should be reconstituted before centrifuge)
2. Using a pipette to take the middle layer for assay (Upper fatty layer should be avoided)
3. Samples with high levels of AFM_1 should be diluted with PBS solution before assaying.

Assay Procedure

1. Format the microplate's wells for each standard or sample to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag and stored at 2-8^{o}C.
2. Wash buffer preparation
   Dilute 10 x washing buffer with distilled water to make 1 x washing buffer by 1:9 ratio. Check if the 10X Wash buffer is at room temperature and the crystal at the bottom is dissolved thoroughly before use.
3. Add 50 µL of Negative control and standard solution (0.01, 0.05, 0.1, 0.25, and 0.5 ppb) or 50 µL of each sample into the assigned well.
4. Add 50 µL of Afla-HRP enzyme conjugate solution (HRP conjugate) to all wells. Swirl the plate gently to mix the content thoroughly. Now the volume of each well is 100 µL.
5. Incubate 30 minutes at room temperature (25-37^{o}C) under dark.
6. Remove liquid from all wells.

7. Flood the wells with at least 300 –350 µL of 1 x washing buffer, and then decant the liquid from all wells.
8. Repeat the step 7 at least three times.
9. Invert and gently pat the plate on absorbent paper towels to remove remaining solution in wells .
10. Add 100 µL of substrate solution to each well and shake gently.
11. Incubate 15 minutes at room temperature (25-37^{o}C) under dark. Blue color develops in the wells with Negative control.
12. Add 100 µL of stop solution to each well and mix gently (Now the volume of each well is 200 µL). Solution will turn from blue into yellow immediately.
13. Read color at OD 450 nm in an ELISA reader within 3-15 minutes after adding the stop solution.

Example of typical plate setup.

NC: Negative Control

C1= 0.01 ppb Aflatoxin M1 standard

C2= 0.05 ppb Aflatoxin M1standard

C3= 0.1 ppb Aflatoxin M1standard

C4= 0.25 ppb Aflatoxin M1 standard

C5= 0.5 ppb Aflatoxin M1 standard

S1, S2, S3, S4, S5 etc.=Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A			S1	S1	S9	S9						
B	NC	NC	S2	S2	S10	S10						
C	C1	C1	S3	S3								
D	C2	C2	S4	S4								
E	C3	C3	S5	S5								
F	C4	C4	S6	S6								
G	C5	C5	S7	S7								
H			S8	S8								

Calculating Results

1. After reading the wells, average the OD of each set of calibrators and samples, and calculate the B/B$_0$% as follows:

$$B/B_0\% = \frac{\text{average OD of Calibrator or sample}}{\text{average OD of Negative Control}} \times 100$$

The %B$_0$ calculation is used to equalize different runs of assay. While the raw OD values of Negative Control, Calibrators, and samples may different from run to run, the %B$_0$ relationship of calibrators and samples to Negative Control should remain fairly constant.

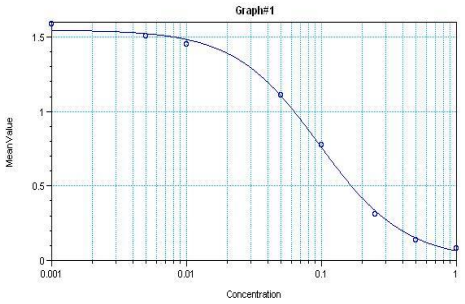
2. Graph the B/B$_0$% of each Calibrator against its Aflatoxin M1 concentration on a semi-log scale
3. Determine the Aflatoxin M1 concentration of each sample by finding its B/B$_0$ value and the corresponding concentration level on the graph.
4. Interpolation of sample concentration is only valid if the B/B$_0$% of sample falls within the range of the B/B$_0$%‘s set by calibrators.
5. Because of the 1:10 dilution of the grain sample in the extraction solution, the sample toxin concentration calculated from the standard curve should multiple 10 folds to get the toxin concentration in the sample

Limit of detection

The detection limit for this assay based on Aflatoxin M1 is 0.005 ppb (ng/mL).

Performance data

1. Aflatoxin M1 standard curve



2. Cross -reactivity

Compound	50% **B/Bo** (ppb)	Cross-Reactivity (%)
Aflatoxin M1	0.09	100

Precision

Well Contents	%CV(n=7)
Negative Control	3.4
0.01 ppb Calibrator	5.0
0.05 ppb Calibrator	4.3
0.1 ppb Calibrator	3.4
0.25 ppb Calibrator	3.2
0.5 ppb Calibrator	1.0

Safety Instructions

The standards of the ELISA kit conatin small amounts of AFM1 and the substrate solution contains tetramethylbenzidine and the stop solution contains diluted HCl. Avoid contactof stopping solution with skin or eyes.