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Aflatoxin M1 ELISA Kit

05/18

(Catalog # E4566-100, 100 assays, Store at 4°C)

I. 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 animals 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 is a competitive ELISA kit, 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.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Aflatoxin M1.
Detection Range: 0.02 – 1.62 ppb (ng/ml)
Sensitivity: 0.02 ppb (ng/ml)
Detection limitation: 0.1 ppb for milk, 0.5 ppb for milk powder
Cross Reactivity: Aflatoxin M1 100%, Aflatoxin B1 30%

III. Sample Type:

Milk, milk powder

IV. Kit Contents:

Components	E4566-100	Part No.
Micro ELISA Plate	8 X 12 Strips	E4566-100-1
Standard (S0 – S5)	1 ml X 6	E4566-100-2
HRP-conjugate	7 ml	E4566-100-3
Antibody	7 ml	E4566-100-4
TMB substrate	12 ml	E4566-100-5
Stop Solution	10 ml	E4566-100-6
Wash Buffer (10X)	50 ml	E4566-100-7
Plate sealers	4	E4566-100-8

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 12 months from the date of shipment. Opened kit may be stable for 1 month at 4°C.

VII. Reagent and Sample Preparation:

Note: Bring all reagents to room temperature (20-25°C) 30 minutes before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes. Finish preparing reagent 10 minutes before the assay.

1. **Wash Buffer (1X):** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals are completely dissolved. Dilute 10 ml of Wash Buffer (10X) into 90 ml deionized water to prepare 100 ml of Wash Buffer (1X). Can be stored at 4℃ for one month.
2. **Standards Concentration:** Ready to use

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

3. Sample Preparation:

Note: The prepared sample maybe stored for up to 3 days at 2-8°C.

- **Milk**
  1. Bring the milk sample to room temperature. Take 50 µl of sample for further analysis. (Dilution factor: 1)
- **Milk Powder**
  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: 8)

FOR RESEARCH USE ONLY! Not to be used on humans.

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VII. Store unused wells back to 2-8°C.
2. 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.
3. Aspirate each well and wash, repeating the process 4 times. Wash by filling each well with 250 µl of **Wash Buffer** 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.
4. Add 100 µl of **TMB Substrate** to each well, mix well. Incubate for 15 minutes at 25°C. Protect from light.
5. Add 50 µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
6. Read result at 450 nm within 5 minutes.

IX. CALCULATION:

To draw a standard curve: Take the absorbency value of standards as y-axis, logarithmic of the concentration of the Aflatoxin M1 standards solution (ppb) as x-axis. The Aflatoxin M1 concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

X. RELATED PRODUCTS:

- Ractopamine ELISA Kit (Cat. No. E4565)
- Clenbuterol ELISA Kit ((Cat. No. E4564)
- Salbutamol (SALB) ELISA Kit (Cat. No. K4209)
- Chloramphenicol (CAP) ELISA Kit (Cat. No. K4230)
- Ciprofloxacin (Cipro) ELISA Kit (Cat. No. E4365)
- Enrofloxacin (ENR) ELISA Kit (Cat. No. E4277)
- Fluoroquinolones ELISA Kit (Cat. No. E4275)