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08/19

Fumonisin B1 ELISA Kit

(Catalog # E4749-100; 96 assays; Storage at 4°C)

I. Introduction:

Fumonisin B1 is the most prevalent member of a family of toxins, known as fumonisins, produced by several species of Fusarium molds, such as Fusarium verticillioides, which occur mainly in maize (corn), wheat and other cereals. Fumonisin B1 is hepatotoxic and nephrotoxic in all animal species tested. The earliest histological change to appear in either the liver or kidney of fumonisin-treated animals is increased apoptosis followed by regenerative cell proliferation. BioVision's fumonisin B1 ELISA Kit is based on Indirect-Competitive-ELISA method. It can detect fumonisin B1 (FB1) in samples, such as grain, peanut, feed, etc. The Microtiter plate provided in this kit has been pre-coated with FB1. During the reaction, FB1 in the samples or standard competes with FB1on the solid phase supporter for sites of FB1 antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each microplate well, and TMB substrate is for color development. There is a negative correlation between the OD value of samples and the concentration of FB1. The concentration of FB1 in the samples can be calculated by comparing the OD of the samples to the standard curve.

II. Applications:

- Detection limit: Corn, feed - 50 ppb, Edible oil - 10 ppb
- Sample recovery rate: Feed - 95%±15%, Corn - 100%±15%, Edible oil - 85%±15%
- Cross-reactivity: Fumonisin B1 (FB1) -100%
- Quantitative measurement of Fumonisin B1 in grain, peanut and feed samples.
- Sensitivity: 0.5 ppb (ng/mL)

III. Sample Type:

- grain, peanut, feed

IV. Kit Contents:

Components	E4749-100	Part Number
ELISA Microplate	96 wells	E4749-100-1
Standard	1 ml X 6	E4749-100-2
HRP Conjugate	5.5 ml	E4749-100-3
Antibody Working Solution	5.5 ml	E4749-100-4
Substrate Reagent A	6 ml	E4749-100-5
Substrate Reagent B	6 ml	E4749-100-6
Stop Solution	6 ml	E4749-100-7
Reconstitution Buffer (10x)	50 ml	E4749-100-8
Wash Buffer (20X)	40 ml	E4749-100-9
Plate Sealer	3	E4749-100-10

V. User Supplied Reagents and Equipment:

- 70% Methanol, Deionized or distilled water
- Microplate reader with 450 nm wavelength filter

VI. Storage Conditions and Reagent Preparation:

Please store the opened kit at 4°C, protect from light and moisture and use within 2 months.
Sample extraction buffer: 70% Methanol. Mix Methanol and deionized water in the ratio 7:3.
Wash Buffer: Dilute the 20x Wash Buffer with deionized water. Mix 20x Wash Buffer into Deionized water in the ratio of 1:19.
Reconstitution buffer: Dilute the 10x Reconstitution Buffer with deionized water. Mix 10x Wash Buffer with deionized water in the ratio of 1:9. Store at 4℃ for a month**.**

Standard Concentration:

Standards	S1	S2	S3	S4	S5	S6
Concentration (ppb)	0	0.5	1.5	4.5	13.5	40.5

Sample pretreatment
Pretreatment of beans, corn and Feed:
1. Weigh 1g /1ml of crushed homogenate in to 50 ml centrifuge tube, add 20 ml of sample extraction buffer, shake well for 5 min, centrifuge at 4000 r/min for 5 min at room temperature.
2. Take 0.1 ml of supernatant and add 1.9 mL of Reconstitution solution. Mix well by gently shaking for 2 minutes.
3. Take 50 µl of sample for analysis.
Note: Sample dilution factor: 100, minimum detection dose: 50 ppb

Pretreatment of edible oil:

1. Take 5 mL of sample into the 50 mL centrifuge tube, add 8 mL of n-hexane and 5 mL of 70% methanol. Mix well for 5 min. Centrifuge at 4000 r/min for 10 min at room temperature.
2. Discard the supernatant, take 100 µL of lower phase liquid, and add 1.9 mL of Reconstitution buffer, mix well.
3. Take 50 µL of sample for analysis.

Note: Sample dilution factor: 20, minimum detection dose: 10 ppb

Assay Protocol:

Note: Restore all reagents and samples to room temperature before use. All the reagents should be mixed thoroughly by gentle mixing before pipetting. Avoid bubble formation.
It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

1. Add 50 µL of **standard or sample** per well, then add 50 µL of **HRP conjugate** and 50 µL of **antibody working solution**, cover the plate sealer, shake for 5 sec gently to mix thoroughly, incubate for 30 min at 25°C.
2. Remove the sealer carefully. Aspirate the liquid. Immediately add 300 µL of **wash buffer (1x)** to each well and wash. Repeat wash procedure for 5 times, 30 s intervals each time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to remove them).
3. Add 50 µL of **Substrate reagent A** to each well, and then add 50 µL of **Substrate reagent B**. Gently mix to mix thoroughly. Incubate at 25°C for 15 min in dark. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min.
4. Add 50 µL of **stop solution** to each well, shake the plate gently to mix thoroughly.
5. Determine the optical density (OD value) of each well at 450 nm (reference wavelength 630 nm) with a microplate reader. Read the plate within 10 min after adding stop solution.

Calculation:

$$\textbf{Absorbance (\%) = A/A0×100\%}$$

A: Average absorbance of standard or sample
A0: Average absorbance of 0 ppb Standard

Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot. Add average absorbance value of sample to standard curve to get corresponding concentration. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.

Related Products:

Zearalenone (ZEN) ELISA Kit (E4276)
Aflatoxin B1 (AFB1) ELISA Kit (K4208)
Aflatoxin M1 ELISA Kit (E4566)

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