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T-2 Toxin ELISA Kit

(Catalog # E4748-100; 96 assays; Storage at 4ºC)

I. Introduction:

T-2 Mycotoxin is a trichothecene mycotoxin. It is a naturally occurring mold byproduct of Fusarium spp. fungus which is toxic to humans and animals. T-2 toxin can cause cell death in human lymphoid T cells and also induces cell membrane damage in B cells. BioVision's T-Toxin ELISA kit is based on the one-step-competitive-ELISA method. This kit can detect T-2 in samples from corn, rice, beans, peanuts, oats, feed, etc. The kit contains Microtiter plate, HRP conjugate, antibody, standard, and other supplementary reagents. The Microtiter plate provided in this kit has been pre-coated with a coupled antigen. During the reaction, T-2 in the samples or standard competes with coupled antigen on the solid phase supporter for binging on an anti-T-2 antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each microplate well and TMB substrate for color development. The OD value of samples is a negative correlation with the concentration of T-2. The concentration of T-2 in the samples can be calculated by comparing the OD of the samples to the standard curve.

II. Applications:

- Sample recovery rate: 110%±15%.
- Sensitivity: 0.05 ppb (ng/ml)
- Quantitative measurement of T-2 Toxin in corn, rice, beans, peanuts, oats, feed samples
- Detection limit: 6 ppb

III. Sample Type:

- corn, rice, beans, peanuts, oats, feed

IV. Kit Contents:

Components	E4748-100	Part Number
ELISA Microplate	96 wells	E4748-100-1
Standard (S1-S6)	1 ml X 6	E4748-100-2
HRP Conjugate	6 ml	E4748-100-3
Antibody Working Solution	6 ml	E4748-100-4
Substrate Reagent A	6 ml	E4748-100-5
Substrate Reagent B	6 ml	E4748-100-6
Stop Solution	6 ml	E4748-100-7
Wash Buffer (20X)	40 ml	E4748-100-8
Plate Sealer	3	E4748-100-9

V. User Supplied Reagents and Equipment:

- Methanol, Deionized water

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: 60% Methanol. Mix Methanol and deionized water in the ratio 3:2.
Wash Buffer: Dilute the 20x Wash Buffer with deionized water. Mix 20x Concentrated Wash Buffer into Deionized water in the ratio of 1:19.
Standard Concentration:

Standards	S1	S2	S3	S4	S5	S6
Concentration (ppb)	0	0.05	0.15	0.45	1.35	4.05

Sample pretreatment
Pretreatment of beans, corn, oats, peanuts, 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 (or filter by quantitative analysis filter paper).
2. Take the supernatant and dilute with deionized water at the ratio of 1:5 (for example, take 1 mL of supernatant and add 5 mL of deionized water).
3. Take 50 µl of sample for analysis.
Note: Sample dilution factor: 120, minimum detection dose: 6 ppb

VII. 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:

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

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.

VIII. Related Products:

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

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