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Deoxynivalenol ELISA Kit

(Catalog # E4750-100; 96 assays; Storage at 4ºC)

I. Introduction:

Deoxynivalenol (DON) also known as Vomitoxin is a type B trichothecene, an epoxy-sesquiterpenoid. This mycotoxin occurs predominantly in grains such as wheat, barley, oats, rye, and corn, and less often in rice, sorghum, and triticale. In higher levels and more advanced stages of contamination, the symptoms linked to vomitoxin are much more severe and include neural problems, immune system deficiencies, hemorrhaging and necrosis of the digestive system, lack of blood production in the spleen and bone marrow, and possible birth defects. BioVision's ELISA Kit is based on Indirect-Competitive ELISA method. It can detect Deoxynivalenol in samples, such as rice, millet, flour and other crops/feed, etc. The micro-plate provided in this kit has been pre-coated with DON. During the reaction, DON in the samples or standard competes with DON on the solid phase supporter for sites of DON antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well, and TMB substrate is for color development. There is a negative correlation between the OD value of samples and the concentration of DON. The concentration of DON in the samples can be calculated by comparing the OD of the samples to the standard curve.

II. Applications:

- Quantitative detection of Deoxynivalenol in rice, millet, flour, and other crops/feed samples.
- **Cross-reactivity:** Deoxynivalenol ($C_{15}H_{20}O_6$) - 100%, 3-Acetyldeoxynivalenol ($C_{17}H_{22}O_6$) - ＜1%
- **Detection limit:** Grain and Feed - 150 ppb
- **Sample recovery rate:** Grain and formula fodder - 85%±15%
- **Sensitivity:** 3 ppb (ng/mL)

III. Sample Type:

rice, millet, flour and other crops/feed

IV. Kit Contents:

Components	E4750-100	Part Number
ELISA Microplate	96 wells	E4750-100-1
Plate Sealer	3	E4750-100-10
Standard	1 ml x 6	E4750-100-2
HRP Conjugate	11 ml	E4750-100-3
Antibody Working Solution	5.5 ml	E4750-100-4
Substrate Reagent A	6 ml	E4750-100-5
Substrate Reagent B	6 ml	E4750-100-6
Stop Solution	6 ml	E4750-100-7
Wash Buffer (20x)	40 ml	E4750-100-8
Reconstitution Buffer (2x)	50 ml	E4750-100-9

V. User Supplied Reagents and Equipment:

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.

Reconstitution Buffer: Dilute the 2x Reconstitution Buffer with deionized water (1:1). Storage for a month at 4 ℃.

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	3	9	27	81	243

Sample pretreatment:

Pretreatment of grain (rice, corn and millet) and Feed:

(1) Weigh 2 g of crushed homogenate into 50 mL EP tube, add 10 mL deionized water, mix well for 5 min, centrifuge at 4000 r/min for 10 min at room temperature

(2) Take 0.1 mL of supernatant, add 0.9 mL of Reconstitution Buffer, mix well

(3) Take 50 µL for detection and analysis.

Note: Sample dilution factor: 50, minimum detection dose: 150 ppb

Pretreatment of corn husk, wheat bran and other strong water absorption feed:

(1) Weigh 2 g of crushed homogenate into 50 mL EP tube, add 20 mL deionized water, mix well for 5 min, centrifuge at 4000 r/min for 10 min at room temperature

(2) Take 0.1 mL of supernatant, add 0.9 mL of Reconstitution Buffer, mix fully

(3) Take 50 µL for detection and analysis.

Note: Sample dilution factor: 100, minimum detection dose: 300 ppb

For the sample containing high level of toxins, it can be diluted by Reconstitution Buffer before determination. For example, take 0.1 mL of the mixed solution, add 0.9 mL of Reconstitution Buffer, and mix well. The final dilution factor of sample is 1000; the minimum detection dose is 3000ppb.

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.

VIII. Related Products:

Total Aflatoxin ELISA Kit (E4747)
Fumonisin B1 ELISA Kit (E4749)
T-2 Toxin ELISA Kit (E4748)

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