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Ochratoxin A (OTA) ELISA Kit

(Catalog # E4746-100; 96 assays; Storage at 4°C)

I. Introduction:

Ochratoxin A (OTA) is a mycotoxin produced by several fungal species including *Aspergillus ochraceus, A. carbonarius, A. niger* and *Penicillium verrucosum*. It is found as a contaminant of barley, corn, wheat, oats and coffee. OTA causes nephrotoxicity and renal tumors in a variety of animal species. BioVision OTA ELISA Kit is based on Indirect-Competitive-ELISA principal. It can detect Ochratoxin A (OTA) in samples, such as grain (rice, peanut, soybean, etc.) and feed. The micro ELISA plate provided in this kit has been pre-coated with OTA. During the reaction, OTA in the samples or standard competes with OTA on the solid phase supporter for sites of OTA 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 OTA. The concentration of OTA in the samples can be calculated by comparing the OD of the samples to the standard curve.

II. Applications:

- **Sensitivity:** 0.1 ppb (ng/mL)
- **Cross-reactivity:** Ochratoxin A (OTA): 100%
- **Detection limit:** Grain: 1 ppb Feed: 2 ppb
- **Sample recovery rate:** Grain/Feed: 85% ± 15%

III. Sample Type:

- Feed
- Grain (rice, peanut, soybean, etc.)

IV. Kit Contents:

Components	E4746-100	Part Number
ELISA Microplate	96 wells	E4746-100-1
Standards (S1 - S6)	1 ml x 6	E4746-100-2
HRP Conjugate	11 ml	E4746-100-3
Antibody Working Solution	5.5 ml	E4746-100-4
Substrate Reagent A	6 ml	E4746-100-5
Substrate Reagent B	6 ml	E4746-100-6
Stop Solution	6 ml	E4746-100-7
Wash Buffer (20x)	40 ml	E4746-100-8
Plate Sealer	3	E4746-100-9

V. User Supplied Reagents and Equipment:

- 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.

Wash Buffer: Dilute 20x Concentrated Wash Buffer with deionized water. Mix 1 ml of 20x Concentrated wash Buffer in 19 ml Deionized water to prepare 20 ml of 1X wash buffer (1:19).

Standard Concentration:

Standards	S1	S2	S3	S4	S5	S6
Concentration (ppb)	0	0.1	0.3	0.9	2.7	8.1

Sample pretreatment procedure:

Pretreatment of grain (rice, corn, millet, etc.)

1. Weigh 2 g of crushed homogenate into 50 mL tube; add 10 mL of 70% methanol, shake well for 5 min, and centrifuge at 4000 r/min for 10 min at room temperature.
2. Take 1 ml of supernatant and add 1 mL of 0.02 M $NaHCO_3$ solution. Mix thoroughly.
3. Take 50 µl for analysis.

Note: Sample dilution factor: 10, minimum detection dose: 1 ppb

Pretreatment of Feed:

1. Weigh 2 g of crushed homogenate into 50 mL EP tube, add 20 mL of 70% methanol, shake well for 5 min, centrifuge at 4000 r/min

for 10 min at room temperature.
2. Take 1 mL of the supernatant and add 1 mL of 0.1 M $NaHCO_3$ Solution. Mix thoroughly.
3. Take 50 µL for detection and analysis.

Note: Sample dilution factor: 20, minimum detection dose: 2 ppb

VII. Assay Protocol:

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 **antibody working solution**, cover the plate sealer, oscillate for 5 sec gently to mix thoroughly, incubate shading light for 30 min at 37°C.
2. Remove the sealer carefully. Aspirate the liquid. Immediately add 300 µL of 1x **wash buffer** to each well and wash. Repeat wash procedure for 5 times, 30 s intervals/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 100 µL of HRP conjugate to each well, incubate in dark for 30 min at 37°C.
4. Repeat wash step as described in step 2.
5. 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 37°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.
6. Add 50 µL of stop solution to each well, shake the plate gently to mix thoroughly.
7. Determine the optical density (OD value) of each well at 450 nm (reference wavelength 630 nm) with a microplate reader. Read the plate within 5 min after adding stop solution.

8. **Calculation:** 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

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

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

VIII. Related Products:

Aflatoxin M1 ELISA Kit (E4566)
Aflatoxin B1 (AFB1) ELISA Kit (K4208)

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