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Zearalenone (ZEN) ELISA Kit

01/17

(Catalog # E4276-100, 100 assays, Store at 4°C)

I. Introduction:

Zearalenone (ZEN), also known as RAL and F-2 mycotoxin, is a potent estrogenic metabolite produced by some Fusarium and Gibberella species. Several Fusarium species produce toxic substances of considerable concern to livestock and poultry producers, namely deoxynivalenol, T-2 toxin, HT-2 toxin, diacetoxyscirpenol (DAS) and zearalenone. Zearalenone is the primary toxin, causing infertility, abortion or other breeding problems, especially in swine. The human and livestock exposure to ZEN through the diet poses health concern due to the onset of several sexual disorders and alterations in the development of sexual apparatus. BioVision's Zearalenone ELISA kit is a competitive ELISA assay for the quantitative measurement of Zearalenone in cereals and compound feeds. The density of color is proportional to the amount of Zearalenone captured from the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Zearalenone.
Detection Range: 0.3 – 24.3 ppb (ng/ml)
Sensitivity: < 0.3 ppb
Detection limit: 6 ppb for cereals and compound feeds.

III. Sample Type:

Cereals and compound feeds

IV. Kit Contents:

Components	E4276-100	Part No.	Cap Color
Micro ELISA Plate	8 X 12 strips	E4276-100-1	-
Standards (S1 – S6)	1.0 ml X 6	E4276-100-2-x	-
High standard (1000 ng/ml)	1.0 ml	E4276-100-3	Black
Antibody working solution	5.5 ml	E4276-100-4	Blue
Enzyme Conjugate	5.5 ml	E4276-100-5	Red
Substrate A solution	6 ml	E4276-100-6	White
Substrate B solution	6 ml	E4276-100-7	Black
Stop Solution	6 ml	E4276-100-8	Yellow
Concentrated Wash Solution (20X)	40 ml	E4276-100-9	White
Adhesive Membrane	1	E4276-100-10	-
Sealed bag	1	E4276-100-11	-

V. User Supplied Reagents and Equipment:

- Reagents: Acetonitrile, N-hexane, Chloroform or Dichloromethane
- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes with disposable tips
- Distilled or deionized water
- Nitrogen-drying device
- 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.

VII. Reagents and Samples Preparation:

Note: Prepare reagents within 30 minutes before the experiment.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Standards:** ready to use.

Tube #	S1	S2	S3	S4	S5	S6
Concentration (ng/ml)	0	0.3	0.9	2.7	8.1	24.3

2. **Wash Buffer:** Dilute 40 ml of the concentrated washing buffer with the distilled or deionized water to 800 ml (or just to the required volume) for using.
3. **Sample Preparation**:

Note: Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles.

- **Cereal, Compound Feed samples**: Weigh 2g crushed samples into 50ml centribuge tube, pipette 8 ml of 90% Acetonitrile solution, mix for 5min and Centrifugal at 4000 rpm at room temperature for 10min. Take 0.5 ml supernatant, add 2 ml deionized water, and mix fully. Take out 50 µl for test. (Dilution times of the sample: 1:20)

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay. Shake the reagent bottles if there is any crystal.

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urve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VII.
2. Add 50 µl **diluted standards** or **samples** into marked well. Add 50 µl **Enzyme Conjugate** into each well, then add 50 µl **antibody working solution** into each well.
3. Oscillate the plate for 5 sec, cover the well and incubate in dark for 30 min at RT (25°C).
4. Discard solution, wash plate 5 times with **1X Wash Solution**. Wash by filling each well with Wash Buffer (250 µl) using a multi-channel pipette or autowasher. Let it soak for 1 min, and then remove all residual wash-liquid from the wells. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent materials.
5. Pipette 50 µl **Substrate A solution**, then pipette 50 µl **Substrate B solution** to each well, oscillate gently for 5 sec, avoid the light preservation for 15 min at RT.
6. Add 50 µl **Stop Solution** to each well and oscillate gently to stop the reaction.
7. Read result at 450 nm within 10 minutes.

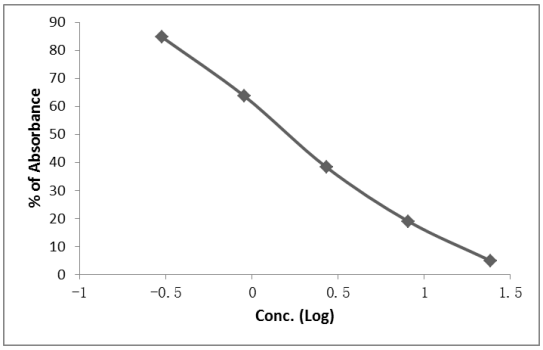
IX. Calculation:

Percentage of absorbance value (%) = A/A_0 X 100%

A: the average (double wells) OD value of the sample or the standard solution; A_0: the average OD value of the 0 ppb standard solution.

To draw the standard curve and calculate, take absorbance percentage of standards as Y-axis, the corresponding log of standards concentration (ppb) as X-axis. Draw the standard semilog curves with X-axis and Y-axis. Take absorbance percentage of samples substitute into standard curve, then can get the corresponding concentration from standard curve; last, Multiplied by the corresponding dilution times is the actual concentration of Sal of samples.

Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.



X. Related Products:

- Sulfonamides residue ELISA Kit (Cat. No. K4207-100)
- Salbutamol (SALB) ELISA Kit (Cat. No. K4209-100)
- Kanamycin ELISA Kit (Cat. No. K4210-100)
- Streptomycin ELISA Kit (Cat. No. K4272-100)
- Fluoroquinolones ELISA Kit (Cat. No. K4205-100)