

Diethylstilbestrol (DES) ELISA Kit

rev 06/18

(Catalog # E4278-100, 100 assays, Store at 4°C)

I. Introduction:

Diethylstilbestrol is a synthetic nonsteroidal estrogen used in the treatment of menopausal and postmenopausal disorders. It was also used formerly as a growth promoter in animals. It is a well-known teratogen and carcinogen, diethylstilbestrol inhibits the hypothalamic-pituitary-gonadal axis, thereby blocking the testicular synthesis of testosterone, lowering plasma testosterone, and inducing a chemical castration. DES was shown to cause clear cell carcinoma, a rare vaginal tumor, in girls and women who had been exposed to this drug in utero. BioVision's Diethylstilbestrol ELISA kit is a competitive ELISA assay for the quantitative measurement of Diethylstilbestrol in animal tissues, honey, milk, egg, milk powder. The density of color is proportional to the amount of Diethylstilbestrol captured from the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Diethylstilbestrol.

Detection Range: 0.02 – 1.62 ppb (ng/ml)

Sensitivity: < 0.02 ppb

Detection limit: 0.08 ppb for tissues.

III. Sample Type:

Animal tissues

IV. Kit Contents:

Components	E4278-100	Part No.	Cap Color
Micro ELISA Plate	8 X 12 strips	E4278-100-1	-
Standards (S1 – S6)	1.0 ml X 6	E4278-100-2-x	-
Antibody working solution	5.5 ml	E4278-100-3	Blue
Enzyme Conjugate	5.5 ml	E4278-100-4	Red
Substrate A solution	6 ml	E4278-100-5	White
Substrate B solution	6 ml	E4278-100-6	Black
Stop Solution	6 ml	E4278-100-7	Yellow
Concentrated Wash Solution (20X)	40 ml	E4278-100-8	White
Adhesive Membrane	1	E4278-100-9	-
Sealed bag	1	E4278-100-10	-

V. User Supplied Reagents and Equipment:

- Reagents: Acetonitrile, Methanol, Acetonitrile, Sodium Hydroxide, Chloroform, Acetone, concentrated phosphoric acid (85%)
- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes with disposable tips
- 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	0.02	0.06	0.18	0.54	1.62

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. **Acetonitrile - Acetone solution:** mix 80 ml of acetonitrile with 20 ml of acetone.
4. **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.

- **Tissues:** Weight 2 g homogeneous tissue samples into the centrifuge tube. Add 6 ml acetonitrile - acetone solution, oscillate for 2 min, centrifuge at room temperature at 4000 rpm for 10 min. Take 3 ml supernatant into a glass tube and blow dry at 50 - 60°C with nitrogen or air. Add 0.5 ml chloroform, oscillate for 20 sec, add 2 ml 2M NaOH oscillate for 30 sec. Centrifuge at RT at 4000 rpm for 10 min. Take 1 ml supernatant, add 200 ul 6M H₃PO₄, oscillate for 20 sec. Add 3 ml acetonitrile, oscillate for 2 min fully, centrifuge at room temperature at 4000 rpm for 10 min. Take supernatant into a glass tube and blow dry at 50 - 60°C with nitrogen or air. Use 1 ml 40% methanol dissolved the dried residue. Take 100 ul of dissolved sample, add 100ul 40% methanol, oscillate for 20 sec. Use 50 µl for the assay. (Dilution factor: 4)

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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. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VII.
2. Add 50 μ l **diluted standards** or **samples** into marked well. Add 50 μ l **Enzyme Conjugate** into each well, then add 50 μ l **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 μ l) 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 μ l **Substrate A solution**, then pipette 50 μ l **Substrate B solution** to each well, oscillate gently for 5 sec, avoid the light preservation for 15 min at RT.
6. Add 50 μ l **Stop Solution** to each well and oscillate gently to stop the reaction.
7. Read result at 450 nm within 10 minutes.

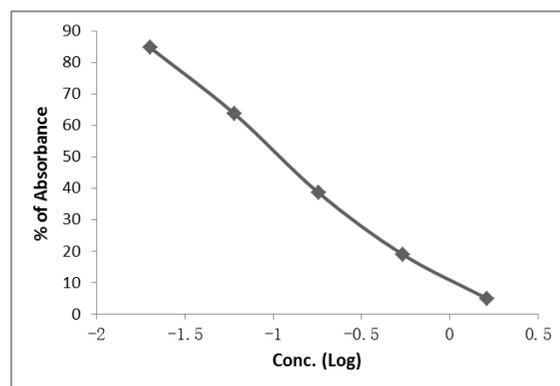
IX. Calculation:

$$\text{Percentage of absorbance value (\%)} = A/A_0 \times 100\%$$

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)

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