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Estradiol (rat) ELISA Kit

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(Catalog # K3831-100, 100 assays; Store at 4°C)

I. Introduction:

Estradiol (E2 or 17β-estradiol) is the predominant female reproductive hormone secreted by the ovaries. Smaller amounts of estradiol are also produced by the adrenal cortex and by the testes. BioVision's rat Estradiol E2 ELISA Kit is based on the standard principle of a sandwich enzyme-linked immunosorbent assay. Rat Estradiol E2 antibody is coated on a 96-well plate. Standards and test samples are added to the wells and Estradiol E2 present in a sample is bound by the immobilized antibody. An HRP-conjugate reagent is added subsequently. After washing away the unbound antibody/HRP conjugates, HRP substrate TMB is used to visualize the HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue product that changes into yellow after adding acidic stop solution. The density of yellow color is proportional to the rat Estradiol E2 captured onto the plate. This ELISA kit shows no species cross-reactivity. Detection Range: 2 – 50 ng/L.

II. Application:

Quantitative detection of Estradiol E2, establishing normal range etc.

III. Specificity:

Rat Estradiol (E2)

IV. Sample Type:

- Serum & plasma (EDTA/Citrate), urine
- Cell culture medium, tissue homogenates and cell lysates

V. Kit Contents:

Components	K3831-100	Part No.
96 wells coated with anti-rat Estradiol antibody, 1 Microplate with 2 adhesive strips	12 strips x 8 wells	K3831-100-1
Rat Estradiol standard (72 ng/L)	0.5 ml	K3831-100-2
HRP-conjugate reagent	6 ml	K3831-100-3
Standard Diluent	1.5 ml	K3831-100-4
Sample Diluent	6 ml	K3831-100-5
Chromogen Solution A	6 ml	K3831-100-6
Chromogen Solution B	6 ml	K3831-100-7
Stop Solution	6 ml	K3831-100-8
Wash Solution (30x stock)	20 ml	K3831-100-9

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended for large number of samples.

VII. Storage Conditions and Reagent Preparation:

Store unopened kit at 4°C for 12 months, protected from light. Once opened, the kit lasts up to 1 month at 4°C. The antibody-coated microplate must be stored in a dry place at 4°C, preferably in a sealed plastic bag. Store the standard at -20°C if the kit is not to be used immediately after receiving. Equilibrate all components to Room Temperature before starting the assay.

- **Preparation of 1x wash solution**: Dilute the 30x concentrated stock 1:30 with distilled water and mix thoroughly. Prepare 0.35 ml of working wash solution for a single wash for each well. The 20 ml stock will make 600 ml of working wash solution.
  Note: If there is precipitation in the wash solution, gently warm to 37°C to dissolve.
- **Rat Estradiol Standard Preparation**: Label 6 tubes with 72, 48, 32, 16, 8 and 4 ng/L of Estradiol. Add 50 µl Standard Diluent to tubes 2-6.

Tube 1: Aliquot 150 µl of the provided Rat Estradiol Standard (72 ng/L)

Tube 2: Add 100 µl from Tube 1 and mix to make 48 ng/L.

Tube 3: Add 100 µl from Tube 2 and mix to make 32 ng/L.

Tube 4: Add 50 µl from Tube 3 and mix to make 16 ng/L

Tube 5: Add 50 µl from Tube 4 and mix to make 8 ng/L

Tube 6: Add 50 µl from Tube 5 and mix to make 4 ng/L

Sample Preparation and Storage:

a. Centrifuge cell culture media, cerebrospinal fluid or urine samples for 20 mins at 2000-3000 rpm to remove particulates. For serum samples, clot in a serum separator tube (20-30 mins) at room temperature. Centrifuge at approximately 2000-3000 rpm for 20 min and use the supernatant. Collect plasma using EDTA or Citrate, mix for 10 mins. Centrifuge for 20 min. at 2000-3000 rpm. For cells and tissues, homogenize in PBS (pH 7.2 – 7.4), spin at top-speed in a table-top centrifuge and collect supernatant. Tissue samples frozen in Liquid-Nitrogen can be ground and used to prepare homogenates.

Notes:

b. For all samples, aliquot and freeze samples at -80°C. Avoid repeated freeze-thaw cycles.
c. Sodium Azide is incompatible with this assay.
d. Sample dilution guidelines: The user needs to estimate the concentration of Estradiol in the sample and select a proper dilution factor so that the diluted Estradiol concentration falls near the middle of the linear regime of the standard curve. Dilute the sample using the provided sample diluent. The sample must be well mixed with the diluent buffer. Several trials may be necessary to optimize sample dilution. Suggested dilution: 1:5. Add 10 µl sample and 40 µl Sample diluent, mix gently without touching the walls of the plate.

VIII. Assay Protocol:

The 96-well plate should not be dry at any time, as drying will inactivate the active components on the plate.

1. Add 50 µl per well of the six rat Estradiol standard solutions in the pre-coated 96-well plate. Add 50 µl sample diluent buffer into the sample control well (Zero well). Add 50 µl each of the 1:5 or properly diluted samples of rat cell culture medium, cell or tissue lysate, urine, serum or plasma (EDTA/Citrate) to each empty well. See "Sample Dilution Guideline" for details.

   Notes:

   a. We recommend that each rat Estradiol standard solution and each sample be measured in duplicate.

   b. We recommend doing a pilot experiment using standards and a small number of samples to validate the assay procedure with the specific samples and optimize the appropriate sample dilution.
2. Seal the plate with the adhesive strip provided and incubate at 37°C for 30 min. Remove the adhesive cover, discard plate contents, and blot the plate onto paper towels or other absorbent material. Do not let the wells completely dry at any time.
3. Add 0.35 ml of prepared working wash solution into each well. Wash plate 5 times, each time leave washing buffer in the wells for 1-2 mins. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. Always drain excess wash solution without drying the wells.
4. Add 50 µl of HRP-conjugate reagent into each well (except the Zero well) and incubate plate at 37°C in dark for 30 min.

   Note: These guidelines are for reference only; the optimal incubation time should be determined by end user. The shades of blue can be seen in the wells with the most concentrated rat Estradiol standard solutions. The other wells might not show any obvious color.
5. Discard the HRP solution and wash the wells as described in Step 3.
6. Add 50 µl of Chromogen solution A and 50 µl of Chromogen solution B into each well. Incubate plate at 37°C in dark for 15 mins. or as required.
7. Add 50 µl of stop solution into each well. The color changes from blue to yellow immediately.
8. Read absorbance at 450 nm in a microplate reader within 15 min. after adding the stop solution.
9. Calculation: Relative $O.D._{450}$ = $O.D._{450}$ of each well – $O.D._{450}$ of Zero well. The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The rat Estradiol concentration of the samples can be interpolated from the standard curve. **Note:** if the samples were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the original concentration in the sample.

Typical Data Obtained from Rat Estradiol E2
(Reaction incubated at 37°C for 30 min.)

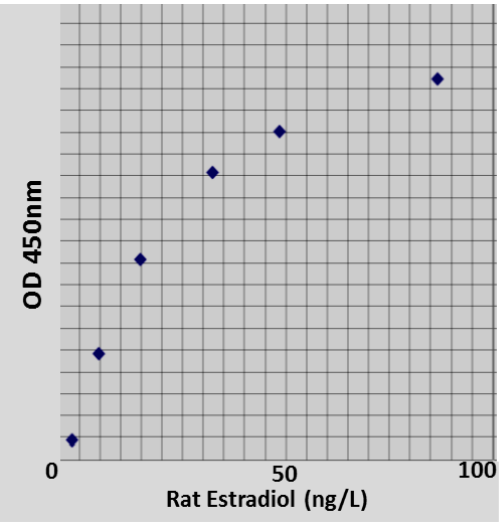

Figure: Standard Curve: This standard curve is for demonstration only. A standard curve must be run with each assay.

IX. RELATED PRODUCTS:

Estradiol (human) ELISA Kit (K3829-100)
Estrogen Sulphotransferase Antibody (3829-100)
Estrogen Sulphotransferase Blocking Peptide (3829BP-50)
Estradiol (mouse) ELISA Kit (K3830-100)
2-Methoxyestradiol (2166)

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