ab285237 – Mouse Estradiol ELISA Kit

For the quantitative measurement of Estradiol in Mouse serum, plasma (Citrate/ EDTA), urine. cell lysates, cell culture supernatants, and tissue homogenates.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285237

Storage and Stability

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

Materials Supplied

Item	Quantity	Storage Condition
96 wells coated with anti-mouse Estradiol antibody, 1 Microplate with 2 adhesive strips	12 strips x 8 wells	+4°C
Mouse Estradiol standard (72 ng/L)	0.5 ml	-20°C
HRP-conjugate reagent	6 ml	+4°C
Standard Diluent	1.5 ml	+4°C
Sample Diluent	6 ml	+4°C
Chromogen Solution A	6 ml	+4°C
Chromogen Solution B	6 ml	+4°C
Stop Solution	6 ml	+4°C
Wash Buffer (30X)	20 ml	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Reagent Preparation

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.

Δ Note: If there is precipitation in the wash solution, gently warm to 37°C to dissolve.

Standard Preparation

Label 6 tubes with 72, 48, 32, 16, 8 & 4 ng/L of Estradiol. Add 50 µl Standard Diluent to tubes 2-6.

- Tube 1: Aliquot 150 µl of the provided Mouse 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

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.

*Δ **Note:** For all samples, aliquot and freeze samples at -80°C. Avoid repeated freeze-thaw cycles.*
*Δ **Note:** Sodium Azide is incompatible with this assay.*
*Δ **Note:** 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.*

Assay Protocol

*Δ **Note:** 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 mouse 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 mouse cell culture medium, cell or tissue lysate, urine, serum or plasma (EDTA/Citrate) to each empty well. See "Sample Dilution Guideline" for details.

*Δ **Note:** We recommend that each mouse Estradiol standard solution and each sample be measured in duplicate.*

*Δ **Note:** 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.

*Δ **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 mouse 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.

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 mouse Estradiol concentration of the samples can be interpolated from the standard curve.

Δ Note: if the samples were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the original concentration in the sample.

Technical Support

For all technical or commercial enquiries please go to:
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