

Product Information

Mouse/Rat Estradiol ELISA

Catalog Number **SE120084**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Estradiol (E2) is the most potent natural estrogen, produced mainly by the ovary, placenta, and in smaller amounts by the adrenal cortex, and in male, the testes. Estradiol is secreted into the blood stream where 98% is bound to sex hormone binding globulin (SHBG). Estrogenic activity is effected via estradiol-receptor complexes which trigger the appropriate response at the follicles, uterus, breast, vagina, urethra, pituitary, hypothalamus, and to a lesser extent the liver and skin. In non-pregnant women with normal menstrual cycles, estradiol secretion follows a cyclic, biphasic pattern with the highest concentration found immediately prior to ovulation. During pregnancy, maternal serum estradiol levels increase considerably, to well above the pre-ovulatory peak levels and high levels are sustained throughout pregnancy. Serum estradiol measurements are a valuable index in evaluating a variety of menstrual dysfunctions such as precocious or delayed puberty in girls, and primary and secondary amenorrhea and menopause. In cases of infertility, serum Estradiol measurements are useful for monitoring induction of ovulation following treatment.

The Mouse/Rat Estradiol ELISA is intended for the quantitative determination of Estradiol (E2) concentration in mouse/rat serum and plasma. It is based on the principle of competitive binding between E2 in the test specimen and E2 Enzyme Conjugate for a constant amount of anti-Estradiol polyclonal antibody. In the incubation, anti-E2 antibody coated wells are incubated with E2 standards, controls, samples, and E2 enzyme conjugate at room temperature for 120 minutes. During the incubation, a fixed amount of HRP-labeled E2 competes with the endogenous E2 in the standard, sample, or quality control serum for a fixed number of binding sites of the specific E2 antibody. E2 Peroxidase Conjugate immunologically bound to the well progressively decreases as the concentration of E2 in the specimen increases. Unbound E2 Peroxidase Conjugate is then removed and the wells are washed.

Next, a solution of TMB Reagent is added and incubated at room temperature for 15 minutes, resulting in the development of blue color. The color development is stopped with the addition of Stop Solution, and the absorbance is measured spectrophotometrically at 450 nm. A standard curve is obtained by plotting the concentration of the standard versus the absorbance.

Components

Materials Provided	96 Tests
Microwells coated with polyclonal anti-Estradiol Antibody	12 x 8 x 1
Estradiol Standards: 6 vials (Ready to use)	0.5 mL
Estradiol Enzyme conjugate Concentrate, 20x, 1 vial	0.7 mL
Assay Diluent , 1 bottle (Ready to use)	12 mL
TMB Reagent, 1bottle (Ready to use)	12 mL
Stop Solution , 1 bottle (Ready to use)	12 mL
Wash Concentrate 20x: 1 Bottle	25 mL

Reagents and Equipment Required but Not Provided.

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA plate reader capable of reading absorbance at 450 nm
5. Absorbent paper or paper towel
6. Graph paper

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at 2–8 °C for 5 days. If storage time exceeds 5 days, store frozen at (–20 °C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to Assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

20× Enzyme Conjugate

Prepare 1× working solution by diluting 20-fold with assay diluent as needed (e.g., 0.1 mL of the 20× Enzyme Conjugate in 1.9 mL of Assay Diluent is sufficient for 20 wells). The diluted conjugate has to be used the same day.

20x Wash Buffer Concentrate

Prepare 1x wash buffer by adding the contents of the bottle to 475 mL of distilled water. Store 1x Wash buffer at room temperature.

Storage/Stability

Store the kit at 2–8 °C. Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit. Do not expose test reagents to heat, sun or strong light.

Procedure

Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.

1. Bring all reagents to room temperature (18–26 °C) before use.
2. Secure the desired number of coated wells in the holder.
3. Dispense 25 µL of standards, specimens, and controls into appropriate wells.
4. Dispense 100 µL of working reagent of Estradiol Enzyme Conjugate into each well.
5. Mix well by placing on shaker for 10–20 seconds.

6. Incubate at room temperature (18–25 °C) for 120 minutes.
7. Remove liquid from all wells. Wash wells three times with 300 µL of 1x Wash buffer. Blot on absorbance paper or paper towel.
8. Dispense 100 µL of TMB Reagent into each well. Gently mix for 10 seconds.
9. Incubate at room temperature (18–25 °C) for 30 minutes.
10. Stop the reaction by adding 50 µL of Stop Solution to each well.
11. Gently mix 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
12. Read absorbance at 450 nm with a microplate reader within 15 minutes.

Results

1. Calculate the mean absorbance value (A_{450}) for each set of reference standards, controls, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in pg/mL on a linear-linear graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis.
3. Use the mean absorbance values for each specimen to determine the corresponding concentration of estradiol in pg/mL from the standard curve.
4. Any values obtained for diluted samples must be further converted by applying the appropriate dilution factor in the calculations.

Each laboratory should establish its own normal range based on the patient population.

Estradiol (pg/mL)	Absorbance (450 nm)
0	2.06
3	1.82
10	1.67
30	1.31
100	0.85
300	0.41

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