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Product Information

Mouse/Rat Estradiol (E2) ELISA kit

Catalog Number: EA100859
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The mouse/rat Estradiol (E2) ELISA Kit is intended for the quantitative determination of Estradiol (E2) concentration in mouse/rat serum and plasma.

Background

Estradiol (E2) is the most potent natural Estrogen, produced mainly by the ovary, placenta, and in smaller amounts by the adrenal cortex, and the male testes. Estradiol is secreted into the blood stream where 98% bound to sex hormone binding globulin (SHBG). Estrogenic activity is affected via estradiol-receptor complexes which trigger the appropriate response at the follicles, uterus, breast, vagina, urethra, hypothalamus, pituitary 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. Estradiol levels have been reported to be increased in patients with feminizing syndromes, gynecomastia and testicular tumors. In cases of infertility, serum Estradiol measurements are useful for monitoring induction of ovulation following treatment.

Principle of the test

The E2 ELISA kit 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 60 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 30 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
1. Microwells coated with polyclonal anti-Estradiol Antibody	12x8x1
2. Estradiol Standards: 6 vials (Ready to use)	0.5 ml
3. Estradiol Enzyme Conjugate Concentrate, 20X, 1 vial	0.7 ml
4. Assay Diluent, 1 bottle (Ready to use)	12 ml
5. TMB Reagent, 1bottle (Ready to use)	12 ml
6. Stop Solution , 1 bottle (Ready to use)	12 ml
7. Wash Concentrate 20X: 1 Bottle	25 ml

Materials and Equipment Required but Not Provided

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

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

Specimen Collection Handling

1. Collect blood specimens and separate the serum immediately.
2. Plasma should be treated with EDTA.
3. Plasma samples may be stored at 2-8°C for up to 8 hours, and should be frozen at -20°C or lower for up to 4 months.
4. Avoid multiple freeze-thaw cycles.
5. Prior to assay, frozen sera should be completely thawed and mixed well.
6. Do not use grossly hemolyzed or lipemic specimens.

Reagent Preparation

1. **20X Enzyme conjugate**: Prepare 1X working solution at 1:20 with assay diluent (e.g. Add 0.1ml of the E2 enzyme conjugate concentrate to 1.9 ml of assay diluent)
2. **Prepare 1X Wash buffer:** Add Wash Concentrate 20X (25 ml) to 475 ml of distilled or deionized water. Store at room temperature (18-26°C).

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C). Gently mix all reagents before use
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed
- It is recommended that standards, control and serum samples be run in duplicate
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities

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 solution 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 60 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.

Calculation of Results

The standard curve is constructed as follows:

1. Check DHEA-s standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.
2. To construct the standard curve, plot the absorbance for DHEA-S standards (Y-axis) versus DHEA-S standard concentrations (X-axis). Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.
4. Any values obtained for diluted samples must be further converted by applying the appropriate dilution factor in the calculations.

Example of Standard Curve

	Estradiol (pg/ml)	Absorbance (450 nm)
Std 1	0	2.06
Std 2	3	1.82
Std 3	10	1.67
Std 4	30	1.31
Std 5	100	0.85
Std 6	300	0.41

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