Human Estradiol ELISA Kit

Catalog number: EK7132

For detection of multiple analytes using one single assay.

This package insert must be read in its entirety before using this product.

For research use only. Not for use in diagnostic procedures.

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antibody and ELISA experts

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3942 B Valley Ave, Pleasanton, CA 94566
888-466-3604 | support@bosterbio.com | www.bosterbio.com

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Catalog Number: EK7132

Size: 96 wells/kit

Sample Type: Serum or plasma

Sensitivity: 10 pg/ml

Assay Range: 10-1000 pg/ml

Storage: Store the kit at 2°C to 8°C. Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit. Do not expose reagent to heat, sun, or strong light. Avoid multiple freeze-thaw cycles (Ships with gel ice, can store for up to 3 days in room temperature. Freeze upon receiving.)

Introduction

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 effected 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, gynaecomastia and testicular tumors. In cases of infertility, serum Estradiol measurements are useful for monitoring induction of ovulation following treatment.

Princple

The Bosterbio E2 ELISA kit is based on the principle of Delayed competitive binding assay between E2 in the test specimen and E2 enzyme conjugate for a constant amount of anti-Estradiol monoclonal antibody epitops (Biotin reagent). In the incubation, anti-E2 antibody biotin reagent, E2 standards, controls, and samples are incubated for 45 minutes at room temperature (RT), then E2 enzyme conjugate is added on the top of the reaction mixture and incubation continues for 45 minutes more. 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 of anti-Estradiol Biotin Reagent and 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 20 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

Kit Components

Description	Quantity
Microwells coated with Streptavidin	12x8x1 Microwells
Estradio Standards	6 vials (ready to use) 0.5 ml

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Biotin Reagent	1 bottle (ready to use) 7 ml
Enzyme Conjugate (20x)	1 vial 0.7ml
Assay Diluent	1 bottle (ready to use) 12ml
TMB Substrate	1 bottle (ready to use) 12ml
Stop Solution	1 bottle (ready to use) 12ml
20X Wash Concentrate	1 bottle 25ml

Standard Concentrations and example data

	OD 450 nm	Estradiol (pg/ml)
Std 1	2.1142	0
Std 2	1.8638	10
Std 3	1.5074	30
Std 4	1.0233	100
Std 5	0.5265	300
Std 6	0.106	1000

Materials Required, but Not Provided

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

Warnings And Precautions

1.The chemotherapeutic drug fulvestrant interferes with the assay. Therefore, we recommend not to use this kit for the determination of estradiol levels in patients undergoing treatment with this agent.
2.For research use only. Not for use in diagnostic procedures.
3.Potential biohazardous materials:
The Standard set contains human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
4.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.
5.Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
6.The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
7. Do not use **sodium azide** as preservative. Sodium azide inhibits HRP enzyme activities.

Specimen Collection Handing

1.Collect blood specimens and separate the serum immediately.
2.Typically, specimens may be stored refrigerated at (2-8°C) for 5 days. For long term storage 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.

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5.Do not use grossly 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 1.9ml of assay diluent)
2.20X Wash buffer: Prepare 1X Wash buffer by adding the contents of the bottle (25ml, 20X) to 475ml of distilled or deionized water. Store at room temperature (20-25°C).

Assay Procedure

1.Bring all reagents to RT (20-25°C) before use.
2.Secure the desired number of coated wells in the holder.
3.Dispense 25µl of standards, specimens and controls into appropriate wells.
4.Dispense 50µl of working solution of Estradiol Biotin Reagent into each well.
5.Mix well by placing on shaker for 10 – 20 seconds.
6.Incubate at (20-25°C) for 45 minutes.
7.Dispense 100µl of Estradiol Enzyme Reagent to all wells. (Note: Add directly on the top of the Biotin)
8.Mix well by placing on shaker for 10 – 20 seconds.
9.Incubate at (20-25°C) for 45 minutes.
10.Remove liquid from all wells. Wash wells three times with 300µL of 1X wash buffer. Blot on absorbance paper or paper towel.
11.Dispense 100µl of TMB Reagent into each well. Incubate at (20-25°C) for 20 minutes.
12.Stop the reaction by adding 50µl of Stop Solution to each well.
13.Gently mix 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
14.Read absorbance at 450 nm with a microplate reader within 15 minutes.

Calculation Of Results

1.Calculate the mean absorbance value (A450) 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.