

CALBIOTECH INC.

Estradiol ELISA

Catalog No. ES071S
(96 tests)

INTENDED USE

For the quantitative determination of Estradiol concentration in human serum.

SUMMARY AND EXPLANATION

Estradiol (E2) is a C18 steroid hormone with a phenolic A ring. This steroid hormone has a molecular weight of 272.4. It is the most potent natural Estrogen, produced mainly by the ovary, placenta, and in smaller amounts by the adrenal cortex, and the male testes. E2 is secreted into the blood stream where 98% of it circulates bound to sex hormone binding globulin (SHBG). To a lesser extent it is bound to other serum proteins such as albumin. Only a tiny fraction circulates as free hormone or in the conjugated form.

Estrogenic activity is effected via estradiol-receptor complexes which trigger the appropriate response at the nuclear level in the target sites. These sites include 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. The rising estradiol concentration is understood to exert a positive feedback influence at the level of the pituitary where it influences the secretion of the gonadotropins, follicle stimulating hormone (FSH), and luteinising hormone (LH), which are essential for follicular maturation and ovulation, respectively. Following ovulation, estradiol levels fall rapidly until the luteal cells become active resulting in a secondary gentle rise and plateau of estradiol in the luteal phase. 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. E2 levels have been reported to be increased in patients with feminizing syndromes, gynaecomastia and testicular tumors.

PRINCIPLE OF THE TEST

The E2 EIA is based on the principle of competitive binding between E2 in the test specimen and E2-HRP conjugate for a constant amount of rabbit anti-Estradiol. In the incubation, goat anti-rabbit IgG-coated wells are incubated with E2 standards, controls, patient samples, Estradiol-HRP Conjugate Reagent and Estradiol reagent. 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. Thus, the amount of 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 washed. Next, a solution of TMB Reagent is added, resulting in the development of blue color. The color development is stopped with the addition of 1N HCl, and the absorbance is measured at 450nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled E2 in the sample. A standard curve is obtained by plotting the concentration of the standard versus the absorbance. The E2 concentration of the specimens and controls run concurrently with the standards can be calculated from the standard curve.

MATERIALS PROVIDED

- Microwells coated with Goat Anti-Rabbit IgG (12x8x1 wells). 96 wells.
- Standard: 6 vials (0.5 mL each). Ready to use.
- Rabbit Anti-E2 Reagent (7 mL) Ready to use.
- Enzyme Conjugate: 1 bottle (12 mL). Ready to use.
- Controls: 1 & 2 (0.5 mL each) Ready to use.
- TMB Substrate: 1 bottle (11 mL). Ready to use.
- Stop Solution: 1 bottle (11 mL). Ready to use.

STORAGE AND STABILITY

1. Store the kit at 2 - 8 °C.

2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials: The calibrator and controls contain 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
2. This test kit is designed for in vitro diagnostic use only.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that serum samples be run in duplicate.
6. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION HANDLING

- Collect blood specimens and separate the serum immediately.
- 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.
- Avoid multiple freeze-thaw cycles.
- Prior to assay, frozen sera should be completely thawed and mixed.
- Do not use grossly lipemic specimens.
- **Please note:** Samples containing sodium azide should not be used in the assay.

REAGENTS PREPARATION

- All reagents should be allowed to reach room temperature (18-25 °C) before use.
- Reconstitute each lyophilized standard with 2.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. Reconstituted standards should be stored sealed and are stable for 30 days at 2-8 °C.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 25 µl of standards, specimens and controls into appropriate wells.
3. Dispense 100 µl of Estradiol-HRP Conjugate Reagent into each well.
4. Dispense 50 µl of rabbit anti-Estradiol(E2) reagent to each well. Thoroughly mix for 30 seconds. It is very important to mix completely.
5. Incubate at room temperature (18-25 °C) for 90 minutes.
6. Rinse and flick the microwells 5 times with *distilled or deionized water. (Please do not use tap water.)*
7. Dispense 100 µl of TMB Reagent into each well. Gently mix for 5 seconds.
8. Incubate at room temperature (18-25 °C) for 20 minutes.
9. Stop the reaction by adding 100 µl of Stop Solution to each well.
10. Gently mix 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
11. Read absorbance at 450 nm with a microtiter well 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. Any values obtained for diluted samples must be

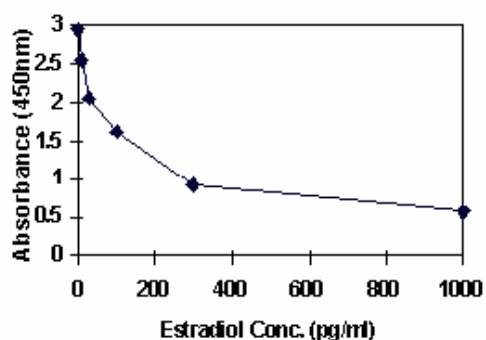
further converted by applying the appropriate dilution factor in the calculations.

EXAMPLE OF THE STANDARD CURVE

Results of a typical standard run with optical density readings at 450nm shown in the Y axis against Estradiol concentrations shown in the X axis.

Note: This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each laboratory must provide its own data and standard curve in each experiment.

Estradiol (pg/ml)	Absorbance (450nm)
0	2.943
10	2.551
30	2.055
100	1.624
300	0.925
1000	0.571



EXPECTED VALUES

Each laboratory should establish its own normal range based on the patient population. The Estradiol ELISA was performed on randomly selected outpatient clinical laboratory samples. The results of these determinations are as follows:

Males:	< 60 pg/ml
Females:	postmenopausal phase < 18 pg/ml
	ovulating, early follicular 30-100 pg/ml
	late follicular 100-400 pg/ml
	luteal phase 60-150 pg/ml
	pregnant, normal up to 35,000 pg/ml
	prepubertal children, normal < 10 pg/ml

SENSITIVITY

The lowest detectable level of Estradiol in this test is 1 pg/ml.

SPECIFICITY

The following materials have been checked for cross reactivity. The percentage indicates cross reactivity at 50% displacement compared to Estradiol.

Data on the cross-reactivity for several endogenous and pharmaceutical steroids are summarized in the following table:

Cross-reactivity (%) = $\frac{\text{Observed Estradiol Concentration}}{\text{Estradiol Concentration}} \times 100$

Steroid Concentration	
<u>Steroid</u>	<u>Cross-Reactivity</u>
Estradiol	100%
Estrone	2.10%
Estriol	1.50%
17a Estradiol	0.30%
Cortisol	<0.01%
Cortisone	<0.01%
Progesterone	<0.01%
Testosterone	<0.01%
DHEA-Sulphate	<0.01%
5a-Dihydrotestosterone	<0.01%

LIMITATIONS OF THE TEST

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

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