

Inter-Assay Precision

Serum	No. of Replicates	Mean pg/ml	Standard deviation	Coefficient of Variation (%)
1	10	9.75	1.25	12.3
2	10	12.8	1.5	11.9
3	10	85.2	6.7	7.8

4. 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:

Steroid	Cross-Reactivity
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%

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**Ultra-Sensitive Estradiol CELISA**

Catalog No. BQ 561S (96 tests)

NAME AND INTENDED USE

The Ultra Sensitive Estradiol (E2) CELISA (Chemiluminescence Enzyme Linked Immunosorbent Assay) is used for the ultra sensitive quantitative measurement of Estradiol (E2) in human serum or plasma.

SUMMARY AND EXPLANATION OF THE TEST

Estradiol (E2) is a C18 steroid hormone with a phenolic A ring. This steroid hormone has a molecular weight of 272.4. Estradiol E2 is the major bioactive 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. The rising estradiol concentration exert a positive feedback influence at the level of the pituitary where it influences the secretion of the gonadotropins, follicle stimulating hormone, and luteinizing hormone, 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. 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. Estradiol EIA kits are designed for the measurement of total Estradiol in human serum or plasma. The assay has high sensitivity well suited to measurements in children and to diagnosis of the menopause.

PRINCIPLE OF THE TEST

The E2 sensitive CELISA kit is based on the principle of competitive binding between E2 in the test specimen and E2 Enzyme conjugate for a constant amount of rabbit anti-Estradiol antibody. E2 standards, controls, patient samples, Estradiol Enzyme Conjugate and rabbit anti-Estradiol antibody are incubated in Goat anti-rabbit IgG-coated wells. During the incubation, a fixed amount of Enzyme 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 washed. Upon the addition of the luminol substrate, the enzyme activity in the enzyme-bound fraction is inversely proportional to the concentration of the E2 in the samples. A standard is prepared relating light unit (RLU) to the concentration of the E2.

Materials provided	96 tests
1. Microwell coated with Goat Anti-Rabbit IgG	6x2x8
2. Estradiol Standards: 8 vials (ready to use)	0.7 ml
3. Rabbit Anti-Estradiol Reagent (ready to use)	7 ml
4. Enzyme Conjugate, 20X, 1 vial	0.35 ml
5. Assay Diluent, 1 bottle	7 ml
6. Luminol substrate, 3X: 1 bottle	4 ml
7. Luminol buffer: 1 bottle	8 ml
8. Wash Concentrate, 20X: 1bottle	25 ml

MATERIAL NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. Microplate luminometer
5. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY

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.

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 kit is designed for Reseach 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 standards, control and 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 AND PREPARATION

1. Serum or EDTA plasma should be used.
2. No special pretreatment of sample is necessary.
3. Serum or plasma samples may be stored at 2-8°C for up to 24 hours, and should be frozen at -10°C or lower for longer periods. Do not use grossly hemolyzed or grossly lipemic specimens.
4. Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

1. 20X Enzyme Conjugate: Prepare 1X working dilution at 1:20 with assay diluent as needed, e.g. 0.1 ml of the stock conjugate in 1.9 ml of assay diluent is sufficient for 20 wells. The diluted conjugate has to be used the same day.
2. 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.
3. 3X Luminol Substrate: Prepare 1X Substrate solution by adding 1 part of Luminol to 2 parts Luminol buffer as needed.

ASSAY PROCEDURE

Prior to assay, bring all reagents to room temperature. Gently mix all reagents before use.

1. Secure the desired number of coated wells in the holder.
2. Add 100 µl of standards, specimens and controls into appropriate wells.
3. Add 50 µl of rabbit anti-Estradiol (E2) to each well.
4. Add 50 µl of Estradiol Enzyme Conjugate to each well.
5. Cover the plate and incubate for 3 hours at room temperature (18-26°C) with shaking.

6. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
7. Add 100 µl of luminol substrate to all wells.
8. Read the relative light units in each well using Luminometer (0.2-1 second integration time) within 5 minutes of substrate addition.

Example of a Standard Data

	Conc.(pg/ml)	RLU
Std 1	0	645037
Std 2	1.25	458663
Std 3	2.5	302157
Std 4	5	212546
Std 5	10	127958
Std 6	25	58443
Std 7	50	34985
Std 8	100	13491

CALCULATION OF RESULTS

The standard curve is constructed as follows:

1. Check Estradiol standard value on each standard vial. This value might vary from lot to lot. Make sure
2. you check the value on every kit. See example of the standard curve.
3. To construct the standard curve, plot the RLU (Relative Light Units) for each Estradiol standard point
4. (vertical axis) versus the Estradiol standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
5. Read the concentration for controls and each unknown sample from the curve. Record the value for
6. each control or unknown sample.

EXPECTED VALUES

Each laboratory should establish its own normal range based on the patient population. The following values could be used as guide line: Males: < 60 pg/ml, Females: postmenopausal phase < 20 pg/ml, ovulating, early follicular 20-150 pg/ml, late follicular 40-350 pg/ml, luteal phase 30-450 pg/ml, pregnant, normal up to 35,000 pg/ml, prepubertal children, normal < 10 pg/ml.

PERFORMANCE CHARACTERISTICS**1. Sensitivity**

The minimum detectable concentration of the Estradiol CELISA assay as measured by 2 SD plus mean of a zero standard is estimated to be 0.4 pg/ml.

2. Correlation with reference CELISA kit

A total of 86 sera were tested by Estradiol and a reference CELISA kit. Results were as follows:

Correlation	Slope	Intercept
0.9	0.98	0.8

3. Precision**Intra-Assay Precision**

Serum	No. of Replicates	Mean pg/ml	Standard deviation	Coefficient of Variation (%)
1	16	9.5	1	10
2	16	13	1.1	8.5
3	16	86.6	4.2	4.6