Estradiol ELISA Kit

(For Human)

Enzyme Immunoassay for the quantification of human Estradiol in serum and plasma.

Catalog number: ARG80839

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

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INTRODUCTION

Estradiol (1,3,5(10)-estratriene-3,17β-diol; 17β-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 Graffian follicle of the female ovary and the placenta, and in smaller amounts by the adrenals, and the male testes.

Estradiol (E2) is secreted into the blood stream where 98% of it circulates bound to sex hormone binding globulin (SHBG) and to a lesser extent to other serum proteins such as albumin. Only a small fraction circulates as free hormone or in the conjugated form. Estrogenic activity is affected via estradiol-receptor complexes which trigger the appropriate response at the nuclear level in the target sites. These sites include the follicles, uterus, breast, vagine, 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 folicular 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 feminising syndromes, gynaecomastia and testicular tumors.

In cases of infertility, serum Estradiol measurements are useful for monitoring induction of ovulation following treatment with, for example, clomiphene citrate, LH-releasing hormone (LH-RH), or exogenous gonadotropins. During ovarian hyperstimulation for in vitro fertislisation (IVF), serum estradiol concentrations are usually monitored daily for optimal timing of human chorionic gonadotropin (hCG) administration and oocyte collection.

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. A highly specific Estradiol antibody has been pre-coated onto a microtiter plate. Estradiol containing samples or standards and an Estradiol-HRP conjugate are given into the wells of the microtiter plate. Enzyme labeled and free Estradiol compete for the antibody binding sites. After incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A substrate solution is added and incubated, resulting in the development of a blue color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The yellow color is measured at 450 nm. The concentration of Estradiol is indirectly proportional to the color intensity of the test sample.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	1 plate	4°C
Standards 0-6 (0, 25, 100, 500, 1000, 2000 pg/ml)	7 X 1 ml (ready to use)	4°C
HRP-Estradiol conjugate	25 ml (ready to use)	4°C
40X Wash buffer	30 ml	4°C
TMB substrate	14 ml	4°C (Protect from light)
STOP solution	14 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the HRP-Estradiol Conjugate before use.
- If crystals are observed in the 40X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.

- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants - Remove particulates by centrifugation and aliquot & store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 40X Wash buffer into distilled water to yield 1X Wash buffer.
- Samples: Dilute 1:100 with standard zero, if samples containing concentrations was higher than the highest standard.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 25 µl of each Standard, Control and samples into appropriate wells.
3. Add 200 µl of HRP-Estradiol conjugate into each well.
4. Cover wells and incubate for 120 minutes at RT.
5. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add 100 µl of TMB Reagent to each well. Incubate for 15 minutes at room temperature in dark.
7. Add 50 µl of Stop Solution to each well.
8. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with

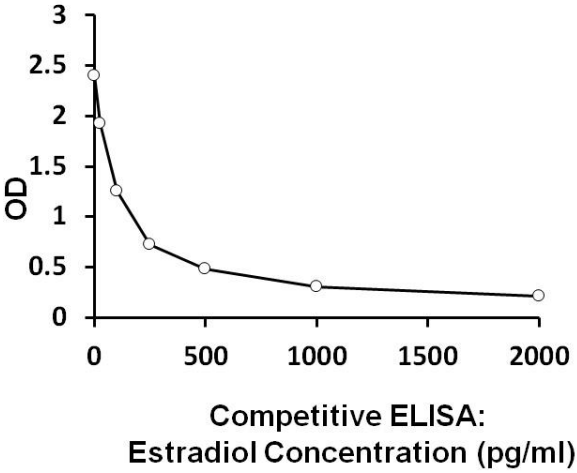
absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The analytical sensitivity was calculated from the mean minus two standard deviations of twenty (20) replicate analyses of Standard 0 and was found to be 9.714 pg/ml.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 4.55% and inter-assay precision was 7.77%.

Specificity

The following substances were tested for cross reactivity of the assay:

Compound	% Cross Reactivity
Estradiol-17ß	100
Androstenedione	0
Androsterone	0
Corticsterone	0
Cortisone	0
Epiandrosterone	0
16-Epiestriol	0
Estradiol-3-sulfate	0
Estradiol-3-glucoronide	0
Estradiol-17α	0
Estriol	0.05
Estriol-16-glucoronide	0
Estrone	0.2
Estrone-3-sulfate	0
Dehydroepiandrosterone	0

Compound	% Cross Reactivity
11-Deoxycortisol	0
21-Deoxycortisol	0
Dihydrotestosterone	0
Dihydroepiandrosterone	0
20-Dihydroprogesterone	0
11-Hydroxyprogesterone	0
17α-Hydroxyprogesterone	0
17α-Pregnenolone	0
17α-Progesterone	0
Pregnanediol	0
Pregnanetriol	0
Pregnenolone	0
Progesterone	0
Testosterone	0

Recovery

86.4-109.4%