Human Estrone ELISA Kit

Enzyme Immunoassay for the quantification of human Estrone in serum and plasma

Catalog number: ARG80843

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INTRODUCTION

Estrone (3-hydroxy-1,3,5 (10)-estratrien-17-one) is beside estradiol and estriol one of the three major naturally occuring estrogens. The estrogens are involved in the development of female sex organs and secondary sex characteristics. Bioassay data indicate that the estrogenic activity of estrone is considerably lower in comparison to estradiol. However, the physiological role of endogenous estrone is not well defined.

Estrone is produced primarily from androstenedione. In premenopausal women, more than 50% of the estrone is secreted by the ovary. In prepubertal children, men and postmenopausal women, the major portion of estrone is derived from peripheral tissue conversion. During the follicular phase of the menstrual cycle the estrone level inreases with a clear peak around day 13. The peak is of short duration and by day 16 of the cycle levels will be low again. A second peak during the luteal phase occurs around day 21 of the cycle. If fertilization does not occur production of estrone decreases again. These changes of estrone concentration are in parallel to that of estradiol. Until the 4 to 6 week of pregnancy, estrone originates primarily from maternal sources such as the ovaries, adrenals, or peripheral conversion thus remaining within the normal values. After the 6 to 10 week of pregnancy the values increase gradually due to placental secretion of estrone. After menopause, estrone levels do not decline as dramatically as estradiol levels. In postmenopausal women estrone is the major estrogen. In males the concentration of E1 has been reported to rise up with age inversely to that of 17-OH-progesterone. In premenopausal women excessive estrone levels can result from the conversion of large amounts of androstenedione produced in polycystic ovary syndrom and ovarian tumors.

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. A highly specific Estrone antibody has been pre-coated onto a microtiter plate. Estrone containing samples or standards and an Estrone -HRP conjugate are given into the wells of the microtiter plate. Enzyme labeled and free Estrone 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 Estrone 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, 15, 30, 90, 270, 810, 2400 pg/ml)	7 X 1 ml (ready to use)	4°C
Control Low	1 ml (ready to use)	4°C
Control High	1 ml (ready to use)	4°C
HRP-Estrone conjugate	14 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-Estrone 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.

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 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. Dilute 30 mL of 40X Wash Solution with 1170 mL deionized water to a final volume of 1200 mL. The diluted 1X Wash Solution is stable for 2 weeks at room temperature.
- **Samples:** If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with Standard 0 and re-assayed as described in Assay Procedure. For the calculation of the concentrations this dilution factor has to be taken into account.

  Example:

  a) For 1:10 dilution: 10 µL sample + 90 µL Standard 0 (mix thoroughly)

  b) For 1:100 dilution: 10 µL 1:10 diluted sample from a) + 90 µL Standard 0 (mix thoroughly).

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 100 µl of HRP-Estradiol conjugate into each well. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Cover wells and incubate for 60 minutes at RT.
5. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1X Wash Buffer (400 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. It is recommended that the wells be read within 10 minutes after adding the Stop Solution.

CALCULATION OF RESULTS

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

2. Using log-log, semi-log or 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.

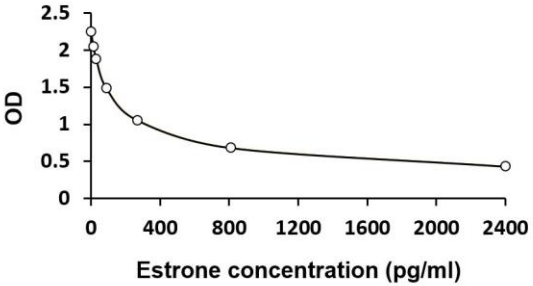
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

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.

5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 2400 pg/mL. For the calculation of the concentrations this dilution factor has to be taken into ac-count.

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 8.1 pg/ml.

The limit of detection (LoD) of the assay is 14.6 pg/mL. The limit of quantification (LoQ) of the assay is 15.8 pg/mL.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 7.08% and inter-assay precision was 8.85%. The CV value of inter-lot precision was 7.36%.

Specificity

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

Compound	Cross reactivity %
17-OH-Progesterone	0.01
Androstenedione	0.04
Corticosterone	0.01
Cortisone	0.39
DHEA	0.01
Estradiol	1.19
Estriol	0.07
Estrone 3-β-D-glucoronide	0.35
Estrone 3-sulfate	0.44
Ethisterone	0.39
Hydrocortisone	0.30
Progesterone	<0.01
Testosterone	0.03

Interference

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results.

Recovery

85-114.6%

Linearity

EDTA plasma: 95.7-113.4

Serum: 88.9-113.4