Human Estrone ELISA Kit

Enzyme Immunoassay for the quantification of Estrone in Human saliva.

Catalog number: ARG82806

Package: 96 wells

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INTRODUCTION

Estrone (E1), also spelled oestrone, is a steroid, a weak estrogen, and a minor female sex hormone. It is one of three major endogenous estrogens, the others being estradiol and estriol. Estrone, as well as the other estrogens, are synthesized from cholesterol and secreted mainly from the gonads, though they can also be formed from adrenal androgens in adipose tissue. Relative to estradiol, both estrone and estriol have far weaker activity as estrogens. Estrone can be converted into estradiol, and serves mainly as a precursor or metabolic intermediate of estradiol. It is both a precursor and metabolite of estradiol.

Estrone is bound approximately 16% to sex hormone-binding globulin (SHBG) and 80% to albumin in the circulation, with the remainder (2.0 to 4.0%) circulating freely or unbound. It has about 24% of the relative binding affinity of estradiol for SHBG. As such, estrone is relatively poorly bound to SHBG.
[Provided by Wikipedia: Estrone]

PRINCIPLE OF THE ASSAY

This assay employs the competitive quantitative enzyme immunoassay technique. A highly specific antibody for Estrone has been pre-coated onto a microplate. Estrone containing samples, Controls 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, the wells are washed with diluted Wash Buffer to remove unbound material. Then TMB substrate is added to the wells and color develops in inversely proportion to the amount of Estrone present in the samples. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450 nm. The concentration of Estrone in the samples is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all other components at 2-8°C. Microtiter wells must be stored at 2-8°C.

Once the foil bag has been opened, care should be taken to close it tightly again.

Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated microplate	8 X 12 strips	4°C
Standards A to F (0, 3, 12.3, 37, 111, 333 pg/mL)	1 mL each (ready to use)	4°C
Control 1 & 2	1 mL each (ready to use)	4°C
Diluent Buffer	3 mL (ready to use)	4°C
Estrone-HRP Conjugate	14 mL (ready to use)	4°C
40X Wash Buffer	30 mL	4°C
TMB substrate	25 mL (ready to use)	4°C (protect from light)
STOP solution	14 mL (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- Saliva collection device
- Mixer or Ultra-Turrax
- Microplate shaker
- Pipettes and pipette tips
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature (21-26°C).
- Remove the number of strips required and return unused strips to the pack and reseal.
- Avoid air bubbles in the wells as this could result in lower binding efficiency and higher CV% of duplicate reading.
- Briefly spin down the all vials before use.
- If crystals are observed in the 40X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not

become completely dry during the assay.

- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Saliva:

1. Collect saliva sample with a centrifuge glass tube (contain a plastic straw).
2. Store at-20°C for at least overnight.
3. Warm up to room temperature and mix carefully. Centrifuge at 3000 g for 5-10 minutes.
4. Use supernatant for the assay.

Note:

1. Samples containing sodium azide should not be used in the assay.
2. Eating, drinking, chewing gums or brushing teeth should be avoided for 30 minutes before sampling.
3. It is recommended to rinse mouth thoroughly with cold water 5 minutes prior to sampling.
4. It is very important that a good clear sample is received. No contamination with food, lipstick, blood (bleeding gums) or other extraneous materials.
5. If there is visible blood contamination the patient specimen, it should be discarded, rinse the sampling device with distilled water, wait for 10 minutes and take a new sample.
6. Specimens should be capped and may be stored for up to one week at 2-8°C prior to assaying. Specimens stored for a longer time (up to 3 months) should be frozen only once at-20°C prior to assay. Thawed samples should be inverted several times prior to testing.
7. Each sample has to be frozen, thawed, and centrifuged at least once in

order to separate the mucins by centrifugation.

8. If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with Diluent Buffer and re-assayed.

REAGENT PREPARATION

- **1X Wash Buffer:** Dilute 40X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 30 mL of 40X Wash Buffer into 1170 mL of distilled water to a final volume of 1200 mL) The 1X Wash Buffer is stable for up to 2 weeks at room temperature.

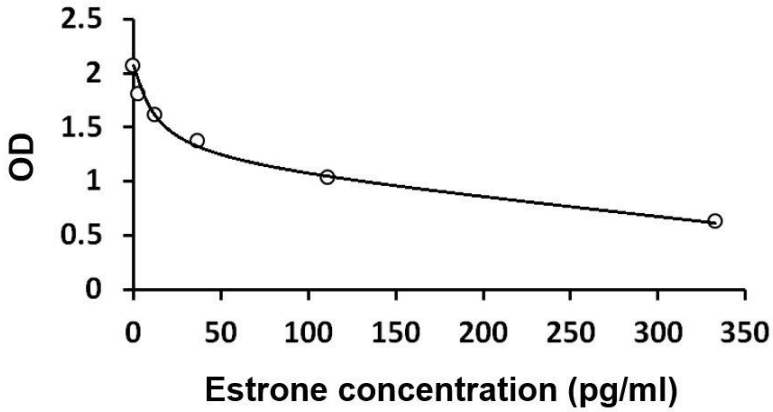
ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 21-26°C) before use. Standards and samples should be assayed in duplicates.

1. Add **100 µL** of **Standards, Controls and prepared samples** into the appropriate wells of the Antibody Coated Microplate.
2. Incubate at **RT** for **30 minutes.**
3. Add **100 µL** of **Estrone-HRP Conjugate** into all wells. Thoroughly mix for **10 seconds**. It is important to have a complete mixing in this step.
4. Incubate at **RT** for **30 minutes** on a microplate shaker.
5. Aspirate each well and wash, repeating the process 4 times for a total **5 washes**. Wash by filling each well with **1× 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 **150 µL** of **TMB Substrate** to each well, including the blank wells. Incubate in the dark for **15 minutes** at **RT** on a microplate shaker.
7. Immediately Add **100 µL** of **Stop Solution** to each well, including the blank wells. The color of the solution should change from blue to yellow.
8. Read the OD with a microplate reader at **450 nm** immediately. (optional: read at 620 nm as reference wavelength) It is recommended reading the absorbance **within 10 minutes** after adding the stop solution.

EXAMPLE OF TYPICAL STANDARD VALUES

The following figures demonstrate typical results with the Human Estrone ELISA kit. One should use the data below for reference only. This data should not be used to interpret actual results.



CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of Controls, standards and 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. 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.
4. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
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 > 333 pg/mL. For the calculation of the concentrations this dilution factor has to be taken into account.
6. For Conversion: pg/mL x 3.69 = pmol/L
7. Reference value:

Population	n	Age (years)	Mean (pg/mL)	Range (pg/mL)
Males	50	16-57	7.69	1.46-20.02
Females	50	19-58	7.37	1.68-29.25

QUALITY ASSURANCE

Sensitivity

The sensitivity of the Human Estrone ELISA kit is 1.073 pg/mL.

Specificity

Substance	Cross Reactivity (%)
Estrone 3-sulfate	52.88
Estradiol	8.65
Estriol	0.32
Progesterone	0.08
17-OH Progesterone	0.05
DHEA-S	0.15
Androstenedione	0.09
4-Androstene-3,17-dione	1.66
Cortisol	ND
DHEA	ND
Testosterone	ND
Cortisone	1.06
Tetrahydrocortisone	0.23
Ethisterone	0.32

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was $\leq$ 9.4% and CV value of inter-assay precision was $\leq$ 14.1%.

Recovery

92.5-106.9%