Human Estradiol ELISA Kit

Enzyme Immunoassay for the quantitative determination of human Estradiol in saliva

Catalog number: ARG80861

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 fertilisation (IVF), serum estradiol concentrations are usually monitored daily for optimal timing of human chorionic gonadotropin (hCG) administration and oocyte collection.

In order to avoid arbitrary results we highly are recommending to always take 5 samples during a 2 hour period and measure the average value of the mixture.

In case of adult women we are recommending to do the sampling around day 21 of the monthly cycle.

Technical advice: In this ELISA kit we are using a special TMB solution which cannot be mixed or exchanged with the substrate solution of any other saliva test kit. This advice might be important for the lab in case of fully or semi-automatic testing.

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	12 strips x 8-well	4°C
HRP-Estradiol Conjugate	26 ml	4°C
Standards 0-5 (0, 1, 5, 10, 50, 100 pg/ml)	6 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
40X Wash Buffer	30 ml	4°C
TMB substrate	25 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.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

Samples containing sodium azide should not be used in the assay. The saliva samples should be completely colorless. Even the slightest red color shows blood contamination. Such blood contamination will give falsely elevated concentration values. In case of visible blood contamination the patient should

discard the sample, rinse the collection device with water, also rinse the mouth with (preferably) cold water, wait for 10 minutes and take a new sample. Do not chew anything during the sampling period. Any pressure on the teeth may result in falsely elevated measurements due to an elevated content of gingival liquid in the saliva sample.

Collection –

1. For the correct collection of saliva we are recommending to only use appropriate devices made from ultrapure polypropylene. Do not use any PE devices or Salivates for sampling; this in most cases will result in significant interferences. Glass tubes can be used as well, but in this case special attention is necessary for excluding any interference caused by the stopper.

2. As food might contain significant amounts of steroid hormones samples preferably should be taken while fasting. If fasting should be a problem at least any food of animal origin (meat or dairy products) should be avoided prior to finalizing the collection. In the morning breakfast should be done only after finalizing the collection procedure. During the day the collection period should be timed just before an anticipated meal. As the steroid hormone secretion in saliva as well in serum shows an obvious dynamic secretion pattern throughout the day it is important to always collect 5 samples during a 2 hour period; this means every 30 minutes one sample. If possible the volume of each single sample should be a minimum of 0.5 ml (better 1 ml). Saliva flow may be stimulated by drinking water. This is allowed and even recommended before and during the collection period. Drinking of water is not allowed during the last 5 minutes before taking the single samples. The typical timing for a morning collection period would be as follows. Wake-up at 6:00 AM, drinking

water and brushing teeth, 1st sample at 6:15 AM, followed by samples at 6:45 AM, 7:15 AM, 7:45 AM, and 8:15 AM, followed by breakfast at 8:25 AM. The typical timing for an afternoon collection period would be like: 1st sample at 5:00 PM, followed by samples at 5:30 PM, 6:00 PM, 6:30 PM, 7:00 PM, followed by dinner at 7:10 PM. Modest variation in the collection timing will not be critical, and the collection timeframe can be extended up to 3 hours.

Storage and Preparation-

Saliva samples in general are stable at ambient temperature for several days. Therefore mailing of such samples by ordinary mail without cooling will not create a problem. Storage at 4°C can be done for a period of up to one week. Whenever possible samples preferable should be kept at a temperature of -20°C. Even repeated thawing and freezing is no problem. Each sample has to be frozen, thawed, and centrifuged at least once anyhow in order to separate the mucins by centrifugation. Upon arrival of the samples in the lab the samples have to stay in the deep freeze at least overnight. Next morning the frozen samples are warmed up to room temperature and mixed carefully. Then the samples have to be centrifuged for 5 to 10 minutes. Now the clear colorless supernatant is easy to pipette. If the sample should show even a slightly reddish color it should be discarded. Otherwise the value most probably will be falsely elevated. Due to the episodic variation of steroid secretion we highly recommend the strategy of multiple sampling. If such a set of multiple samples have to be tested the lab (after at least one freezing, thawing, and centrifugation cycle) has to mix the aliquots of the 5 single samples in a separate sampling device and perform the testing from this mixture.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 40X Wash buffer into distilled water to yield 1X Wash buffer. The diluted Wash Solution is stable for 2 weeks at room temperature.
- **Sample**: If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with 0.9 % NaCl 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) Dilution 1:10: 10 µl saliva + 90 µl 0.9 % NaCl (mix thoroughly)

  b) Dilution 1:100: 10 µl of dilution a + 90 µl 0.9 % NaCl (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 100 µl Standards, controls and samples in duplicate into the appropriate wells. Incubate for 30 minutes at RT.
3. Add 200 µl HRP-Estradiol antibody into each well. Mix thoroughly for 10 seconds and then incubate for 120 minutes at RT.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 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.

5. Add 200 µl of TMB mixture to each well. Incubate for 30 minutes at room temperature in dark.
6. Add 100 µl of Stop Solution to each well.
7. 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.

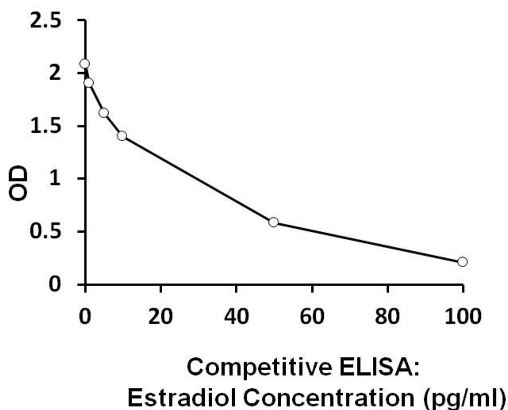
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

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. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

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 plus two standard deviations of twenty (20) replicate analyses of Standard 0 is 0.6 pg/ml.

Specificity

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

Steroid	Cross Reaction (%)
Estradiol-17 beta	100
Estrone	6.86
Estriol	2.27
Androstenedione	0
Androsterone	0

Corticosterone	0
Cortisone	0
Epi-androsterone	0
16-Epiestriol	0
Estadiol-3-sulfate	0
Estradiol-3-glucoronide	0
Estradiol-17 alpha	0
Estriol-16-glucoronide	0
Estrone-3-sulfate	0
Dehydroepiandrosterone	0
11-Desoxycortisol	0
21-Desoxycortisol	0
Dihydrotestosterone	0
Dihydroepiandrosterone	0
20-Dihydroprogesterone	0
11-Hydroxyprogesterone	0
17 alpha-Hydroxyprogesterone	0.003
17 alpha-Pregnenolone	0
17 alpha Progesterone	0
Pregnanediol	0
Pregnantriol	0
Pregnenolone	0
Progesterone	0
Testosterone	0.033
Fulvestrant	0.9

Intra-assay and inter-assay precision

The CV value of intra-assay precision was 4.63% and the CV value of inter-assay precision was 6.55%.

Recovery

85.4-112%