Estradiol (Human) ELISA Kit

Catalog Number KA0234

96 assays

Version: 03

Intended for research use only

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Table of Contents

Introduction

Intended Use

For the quantitative determination of Estradiol (E2) concentration in human serum.

Background

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 ovary, placenta, and in smaller amounts by the adrenal cortex, and the male testes (1,2,3).

Estradiol (E2) is secreted into the blood stream where 98% of it circulates bound to sex hormone binding globulin (SHBG). To a lesser extent it is bound to other serum proteins such as albumin. Only a tiny fraction circulates as free hormone or in the conjugated form (4,5). Estrogenic activity is effected via estradiol-receptor complexes which trigger the appropriate response at the nuclear level in the target sites. These sites include 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 (6,7). 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 luteinizing hormone (LH), which are essential for follicular maturation and ovulation, respectively (8,9). 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 (10).

Serum Estradiol measurements are a valuable index in evaluating a variety of menstrual dysfunctions such as precocious or delayed puberty in girls (11) and primary and secondary amenorrhea and menopause (12). Estradiol levels have been reported to be increased in patients with feminizing syndromes (14), gynaecomastia (15) and testicular tumors (16).

sIn 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 (17,18). During ovarian hyperstimulation for in vitro fertilization (IVF), serum estradiol concentrations are usually monitored daily for optimal timing of human chorionic gonadotropin (hCG) administration and oocyte collection (19).

Principle of the Assay

The E2 EIA is based on the principle of competitive binding between E2 in the test specimen and E2-HRP conjugate for a constant amount of rabbit anti-Estradiol. In the incubation, goat anti-rabbit IgG-coated wells are incubated with 25 µl E2 standards, controls, patient samples, 100 µl Estradiol-HRP Conjugate Reagent and 50 µl rabbit anti-Estradiol reagent at room temperature (18-25℃) for 90 minutes. During the incubation, a fixed amount of HRP-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. Thus, the amount of 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. Next, a solution of TMB Reagent is added and incubated at room temperature for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of 1N HCl, and the absorbance is measured spectrophotometrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled E2 in the sample. A standard curve is obtained by plotting the concentration of the standard versus the absorbance. The E2 concentration of the specimens and controls run concurrently with the standards can be calculated from the standard curve.

General Information

Materials Supplied

List of component

Component	Amount
Goat Anti-Rabbit IgG-coated microtiter wells	96 wells
Estradiol Reference Standards: 0, 10, 30, 100, 300, and 1000 pg/ml. Liquid, ready to use.	0.5 ml each
Rabbit Anti-Estradiol Reagent (pink color)	7 ml
Estradiol-HRP Conjugate Reagent (blue color)	12 ml
Estradiol Control 1, Liquid, Ready to use	0.5 ml
Estradiol Control 2, Liquid, Ready to use	0.5 ml
TMB Reagent (One-Step)	11 ml
Stop Solution (1N HCl)	11 ml

Storage Instruction

Unopened test kits should be stored at 2-8℃ upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above.

Materials Required but Not Supplied

- ✓ Precision pipettes: 25 µl , 50 µl, 100 µl, 200 µl, and 1.0 ml.
- ✓ Disposable pipette tips.
- ✓ Distilled and deionized water.
- ✓ Vortex mixer or equivalent.
- ✓ Absorbent paper or paper towel.
- ✓ Linear-linear graph paper.
- ✓ A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 O.D. at 450 nm wavelength is acceptable for use in absorbance measurement.

Precautions for Use

1. Test methods are not available which can offer complete assurance that Hepatitis B virus, Human Immunodeficiency Virus (HIV/HTLV-III/LAV), or other infectious agents are absent from the reagents in this kit. Therefore, all human blood products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation, where it exists (e.g., USA Center for Disease Control/National Institute of Health Manual, “Biosafety in Microbiological and Biomedical Laboratories,” 1984)(22).
2. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
4. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

Assay Protocol

Sample Preparation

1. Only human serum should be used.
2. No special pretreatment of sample is necessary.
3. Serum samples may be stored at 2-8℃ for up to 24 hours, and should be frozen at –10℃ or lower for longer periods. Do not use grossly hemolyzed or grossly lipemic specimens.
4. Please note: Samples containing sodium azide should not be used in the assay.
5. Samples with expected Estradiol concentrations over 1000 ng/ml may be quantitated by dilution with diluent available from Abnova, Inc.

Assay Procedure

1. All reagents should be brought to room temperature (18-25℃) before use.
2. Secure the desired number of coated wells in the holder.
3. Dispense 25 µl of standards, specimens and controls into appropriate wells.
4. Dispense 100 µl of Estradiol-HRP Conjugate Reagent into each well.
5. Dispense 50 µl of rabbit anti-Estradiol(E2) reagent to each well.
6. Thoroughly mix for 30 seconds. It is very important to mix them completely.
7. Incubate at room temperature (18-25℃) for 90 minutes.
8. Rinse and flick the microwells 5 times with distilled or deionized water. (Please do not use tap water.)
9. Dispense 100 µl of TMB Reagent into each well. Gently mix for 10 seconds.
10. Incubate at room temperature (18-25℃) for 20 minutes.
11. Stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
13. Read absorbance at 450 nm with a microtiter well reader within 15 minutes.

- **Quality Control**

Good laboratory practice requires that controls are run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. We recommend using Bio-Rad Lyphochek Immunoassay Control Sera as controls. Abnova Estradiol EIA kit also provides with internal controls, Level 1 and 2.

Data Analysis

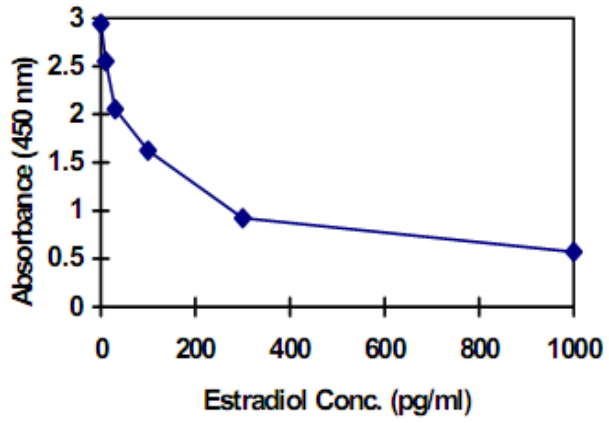
Calculation of Results

1. Calculate the mean absorbance value (A_{450}) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in pg/ml on a linear-linear graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
3. Use the mean absorbance values for each specimen to determine the corresponding concentration of Estradiol in pg/ml from the standard curve.
4. Any values obtained for diluted samples must be further converted by applying the appropriate dilution factor in the calculations.

- **Example of Standard Curve**

Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against Estradiol concentrations shown in the X axis. Note: This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each laboratory must provide its own data and standard curve in each experiment.

Estradiol (pg/ml)	Absorbance (450 nm)
0	2.943
10	2.551
30	2.055
100	1.624
300	0.925
1000	0.571



- **Eexpected Values and Sensitivity**

Each laboratory should establish its own normal range based on the patient population. Abnova Estradiol EIA was performed on randomly selected outpatient clinical laboratory samples. The results of these determinations are as follows:

Males:		< 60 pg/ml
Females:	postmenopausal phase	< 18 pg/ml
	ovulating, early follicular	30-100 pg/ml
	late follicular	100-400 pg/ml
	luteal phase	60-150 pg/ml
	pregnant, normal	up to 35,000 pg/ml
	prepubertal children, normal	< 10 pg/ml

Performance Characteristics

- **Sensitivity**

The minimum detectable concentration of the Abnova Estradiol ELISA assay as measured by 2 SD from the mean of a zero standard is estimated to be 10 pg/ml..

- **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:

✓ Cross-reactivity (%) = Observed Estradiol Concentration / Steroid Concentration × 100.

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%

- **Precision**

Within-run precision was determined by replicate determinations of four different serum samples in one assay. Within-assay variability is shown below:

Samples	1	2	3	4
# Replicates	24	24	24	24
Mean Estradiol (pg/ml)	13	73	247	633
S.D.	3	7	10	31
C.V. (%)	24.1	10.3	4.1	4.9

Between-run precision was determined by replicate measurements of six different serum samples over a series of individually calibrated assays. Between-assay variability is shown below:

Samples	1	2	3	4
# Replicates	20	20	20	20
Mean Estradiol (pg/ml)	14	82	264	691
S.D.	4	8	17	45
C.V. (%)	26.7	10.3	6.4	6.6

- **Spiking Recovery**

Various patient serum samples of known Estradiol levels were combined and assayed in duplicate. The mean recovery was 101.3%.

PAIR NO.	EXPECTED [Estradiol] (pg/ml)	OBSERVED [Estradiol] (pg/ml)	% RECOVERY
1	580	599	103.3
2	810	733	90.4
3	280	249	89.2
4	265	285	107.5
5	81	91	112.6
6	62	67	107.3
7	18	18	98.7

Resources

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Plate Layout

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11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
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