

INTENDED USE

Competitive immunoenzymatic colorimetric method for quantitative determination of Estradiol concentration in human serum or plasma. Estradiol ELISA kit is intended for laboratory use only.

1. CLINICAL SIGNIFICANCE

Estradiol is a sex hormone. It represents the major estrogen in humans. Estradiol has not only a critical impact on reproductive and sexual functioning, but also affects other organs including bone structure. During the reproductive years most Estradiol in women is produced by the ovaries, smaller amounts of Estradiol are also produced by the adrenal cortex. In men, the testes produce Estradiol. In plasma, Estradiol is largely bound to sex hormone binding globulin (SHBG), also to albumin, only a fraction is free and biologically active. Serum Estradiol measurement in women reflect primarily the activity of the ovaries. During pregnancy estrogen levels, including Estradiol, rise steadily towards term. Estradiol increases due to placental production. In adult premenopausal women, ovarian production of Estradiol is stimulated by luteinizing hormone (LH) and the follicle-stimulating hormone (FSH) during the menstrual cycle. In adult women, Estradiol levels are measured in the evaluation of fertility and menstrual irregularities, and to monitor ovarian follicular function during induction of ovulation. In the female, Estradiol acts as a growth hormone for tissue of the reproductive organs. The development of secondary sexual characteristics in women is driven by Estradiol. Estradiol is involved also in man fertility. Estradiol regulates the bone maintenance. Post-menopause women experience an accelerated loss of bone mass due to a relative estrogen deficiency.

Estradiol affects the production of multiple proteins including lipoproteins, binding proteins, and proteins responsible for blood clotting. Estrogens have been found to have neuroprotective function. The Estradiol, for his activities, is involved in some types of cancer such as breast cancer and cancer of the uterine lining. In addition, there are several benign gynecologic conditions that are dependent on estrogen such as endometriosis, leiomyomata uteri, and uterine bleeding.

2. PRINCIPLE

Estradiol in the sample competes with the antigenic Estradiol conjugated with horseradish peroxidase (HRP) for binding to the limited number of antibodies anti-Estradiol coated on the microplate (solid phase).

After incubation, the bound/free separation is performed by a simple solid-phase washing. Then the enzyme HRP in the bound-fraction reacts with the Substrate (H_2O_2) and the TMB Substrate and develops a blu color that changes into yellow when the Stop Solution (H_2SO_4) is added. The color intensity is inversely proportional to the Estradiol concentration in the sample. Estradiol concentration in the sample is calculated through a calibration curve.

3. REAGENT, MATERIAL AND INSTRUMENTATION**1. Estradiol Calibrators (6 Vial)**

CAL0 (1 mL), CAL1 (0.5 mL), CAL2 (0.5 mL), CAL3 (0.5 mL), CAL4 (0.5 mL), CAL5 (0.5 mL)

2. Estradiol Control (1 vial, 0.5 mL)**3. Conjugate (1 vial, 22 mL)****4. Coated Microplate (1 microplate breakable)****5. 10X Conc. Wash Solution (1 vial, 50 mL)****6. TMB Substrate (1 vial, 15 mL)****7. Stop Solution (1 vial, 15 mL)**

3.2. Reagents necessary not supplied in the kit

Distilled water

3.3. Auxiliary materials and instrumentation

Automatic dispenser. Microplates reader (450 nm, 620-630 nm)

Notes

Store all reagents between 2-8°C in the dark.

Open the bag of 4 (Coated Microplate) only when it is at room temperature and close it immediately after use; once opened, it is stable until expiry date of the kit.

4. WARNINGS

- This kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- Some reagents contain small amounts of Proclin 300 as preservatives. Avoid the contact with skin or mucosa.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested.
- Avoid the exposure of reagent TMB/H₂O₂ to directed sunlight, metals or oxidants. Do not freeze the solution.
- This method allows the determination of Estradiol from 20 pg/mL to 2000 pg/mL.
- The clinical significance of Estradiol determination can be invalidated if the patient was treated with cortisone or natural or synthetic steroids.

5. PRECAUTIONS

- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of the reagents.

- Samples microbiologically contaminated, highly lipemic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

6. PROCEDURE

6.1. Preparation of the Calibrators (C0...C5)

The Calibrators are ready to use and have the following concentration of Estradiol:

	C0	C1	C2	C3	C4	C5
pg/mL	0	20	120	300	600	2000

The Calibrators are stable until the expiry date printed on the label. Once opened, the standards are stable six months at 2-8°C.

6.2. Preparation of the Conjugate

The conjugate is ready to use. Mix gently, for 5 minutes, with a rotating mixer. Once opened, it is stable six months at 2 ÷ 8°C

6.3. Preparation of Wash Solution

Dilute the content of the vial "Conc. Wash Solution 10X" with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect the 1:10 dilution ratio. The diluted wash solution is stable for 30 days at 2÷8°C. In concentrated wash solution is possible to observe the presence of crystals, in this case mix at room temperature until complete dissolution of crystals, for greater accuracy dilute the whole bottle of concentrated wash solution to 500 mL taking care also to transfer crystals, then mix until crystals are completely dissolved.

6.4. Preparation of the sample

The determination of Estradiol can be performed in human serum or plasma. Store the sample at -20°C if the determination is not performed on the same day of the sample collection. Avoid repetitive freezing and thawing of samples. Before using, mix gently, for 5 minutes, with a rotating mixer. The Control is ready for use.

6.5. Procedure

- Allow all reagents to reach room temperature (22-28°C).
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C0-C5), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample/ Control	Blank
Calibrator C0-C5	25 µL		
Sample/ Control		25 µL	
Conjugate	200 µL	200 µL	

Incubate 2 h at +37°C. Remove the contents from each well, wash the wells three times with 300 µL of diluted wash solution			
TMB Substrate	100 µL	100 µL	100 µL
Incubate at room temperature (22-28°C) for 30 minutes in the dark.			
Stop Solution	100 µL	100 µL	100 µL
Shake gently the microplate. Read the absorbance (E) at 450 nm against reference wavelength of 620-630 nm or against blank within 5 minutes			

7. QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of Estradiol for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the calibration curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8. RESULTS

8.1. Mean Absorbance

Calculate the mean of the absorbance (E_m) for each point of the calibration curve (C0-C5) and of each sample

8.2. Calibration curve

Plot the mean value of absorbance (E_m) of the calibrators (C0-C5) against concentration. Draw the best-fit curve through the plotted points (es: Four Parameter Logistic).

8.3. Calculation of results

Interpolate the values of the samples on the calibration curve to obtain the corresponding values of the concentrations expressed in pg/mL.

9. REFERENCE VALUES

The serum Estradiol reference values are:

		pg/mL
WOMEN	Follicular phase	30 – 100
	Ovulatory peak	130 – 350
	Luteinic phase	50 – 180
	Menopause	< 60
CHILDREN		< 40
MEN		< 60

10. PERFORMANCE AND CHARACTERISTICS

10.1. Precision

10.1.1. Intra Assay Variation

Within run variation was determined by replicate measurements (10x) of three different human sera in one assay. The within assay variability is $\leq 9\%$.

10.1.2. Inter Assay Variation

Between run variation was determined by replicate measurements of three different human sera in different lots. The between assay variability is $\leq 10\%$.

10.2. Accuracy

The dilution test conducted with high concentration samples of Estradiol gave an average recovery value (\pm SD) of $95.69\% \pm 7.74\%$ with reference to the original concentrations.

The recovery of 120 – 240 – 480 – 960 pg/mL of Estradiol added to samples gave an average value (\pm SD) of $101.09\% \pm 5.42\%$ with reference to the original concentrations.

10.3. Sensitivity

The lowest detectable concentration of Estradiol that can be distinguished from the calibrator zero is 8.68 pg/mL at the 95% confidence limit.

10.4. Specificity

The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

Substance	Cross-Reactivity
Estradiol	100%
Estrone	2%
Estriol	0.39%
Testosterone	0.02%
Cortisol	$< 7 \times 10^{-3}\%$
Progesterone	$< 3 \times 10^{-4}\%$
Dhea-s	$< 1 \times 10^{-4}\%$

10.5. Correlation with a commercial kit

Estradiol ELISA was compared to another commercially available Estradiol assay.

16 serum samples were analysed in both test systems.

The linear regression curve is: $(\text{Estradiol Dia}) = 1.03 * (\text{Estradiol Ref}) - 12.96$ $r^2 = 0.996$

11. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

PUBLICATIONS

- Joshi,U.M., Steroids 34 (1) 35 (1979)
- D.Exley and R. Abuknesha Febs Letters 91,(2)162 (1978)
- Ismail A.A, et al J.Clin.Endocrin.Metab. 34,177-184 (1972)
- Rajkowski,K.M, et al Steroids 29-5 (1977)

BIOGENIX INC. PVT. LTD.

Factory: B - 19/A, S.I.L Ancillary Estate, Amausi Industrial Area, Nadarganj, Kanpur Road,
Lucknow - 226008 (U.P.), India

Email: biogenix2007@yahoo.com, info@biogenixinc.com

Website: www.biogenixinc.com

Customer care no: +919140971443

