ESTRADIOL

Enzyme-immunoassay for the quantitative determination of Estradiol in serum or plasma

REF GD7260 00

IVD

INDICATION

Estradiol (17β-estradiol) is a sex hormone and 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, it is produced by the testes. In plasma estradiol is largely bound to sex hormone binding globulin, 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 estradiol production is stimulated by the interactions of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) throughout 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 and for the development of secondary sexual characteristics.
Estradiol is also involved in man fertility.
Estradiol regulates the bone maintenance. Women who past the menopause 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. Estrogen is considered an oncogene as it supports certain cancers, notably breast cancer and cancer of the uterine lining. In addition there are several benign gynecologic conditions that are dependent on estrogen such as endometriosis.

PRINCIPLE OF THE ASSAY

This test is based on "one step" competition enzyme immunoassay principle (ELISA). Tested specimen is placed into the microwells coated by specific anti-Estradiol antibodies simultaneously with Estradiol conjugated to Horseradish peroxidase (HRP). Estradiol from the specimen competes with the conjugated antigen for coated antibodies. After washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and quantified by addition of chromogen-substrate solution. The developed colour, detected at 450 nm, is inversely related to the quantity of Estradiol present in the specimen.
Estradiol concentration in the sample is calculated based on a series of standards.

KIT CONTENT

1. **Reagent A – Microplate**
   12x8 strips.
   8 wells breakable strips, coated with anti-Estradiol antibody. The strips are assembled on a plastic frame and contained in a sealed bag with desiccant. Bring the strips to room temperature before use, to prevent any moisture formation inside the bag.

2. **Reagent B – Enzymatic Tracer**
   1 vial of 22 ml.
   Estradiol, conjugated with Horseradish peroxidase (HRP).

3. **Reagent C – Washing Solution 10x**
   1 vial of 50 ml.
   Concentrated solution to be diluted 1:10 with distilled water.
   It contains Phosphate buffer 0.2 M, Proclin 0.002%.

4. **Reagent D/E – Chromogen/Substrate**
   1 vial of 15 ml.
   Ready to use solution containing Tetramethylbenzidine (TMB) 0.25 g/l.
   Avoid any skin contact and light exposure.

5. **Reagent F – Stop Solution**
   1 vial of 15 ml.
   Ready to use solution containing Sulphuric acid 0.15 M.
   Avoid any skin contact.

6. **Estradiol Standards**
   6 vials: S_0: 1 ml, S_1-S_5: 0.5 ml.
   Ready to use liquids containing Estradiol approximately at the following concentrations:
   S_0: 0 pg/ml, **S_1**: 20 pg/ml, **S_2**: 120 pg/ml,
   S_3: 300 pg/ml, **S_4**: 600 pg/ml, **S_5**: 2000 pg/ml.
   Actual concentrations to be used for calculation are stated on the labels of the vials.

7. **Cardboard sealers**
   2 cardboard sealers to be used to cover the plate during the incubations.

8. **Package insert:** instruction for use GD7260 00 it/ing.

MICROBIOLOGICAL STATE AND CLEANING GRADE

1. All the materials of human origin resulted negative to HbsAg, HIV 1&2 and HCV FDA approved tests. Anyhow, as no test can guarantee the absolute absence of infective agents, handle reagents as potentially infected, especially standards, controls and samples. All objects come in direct contact with samples and all residuals of the assay should be treated or eliminated as potentially infected. Best procedures for inactivation are treatments with autoclave at 121°C for 30 minutes or with sodium hypochlorite at a final concentration of 2.5 % for 24 hours.
2. Avoid any contact with skin and mucous membrane, in particular for Stop Solution.
3. Use protective disposable talk-free gloves.
4. Avoid contaminating reagents when taking them from the vials. We recommend to use automatic pipettes with disposable tips. When dispensing reagents, do not touch with tips the wall of wells in order to avoid cross-contaminations.
5. For the washing step, follow carefully the indications reported in "WASHING INSTRUCTION".
6. Avoid the substrate/chromogen to come in contact with oxidizing agents or metallic surfaces; avoid intense light exposure during incubation or reagent preparation.

STORAGE AND STABILITY OF THE KIT

1. The kit has to be stored at 2-8 °C and used before the expiry date stated on the label.
2. Unused strips have to be placed in the bag containing the desiccant and firmly sealed before restore at 2-8 °C. After opening the strips are stable up to the expiry date stated on the label.
3. All other reagents can be repeatedly used up to exhaustion if stored at 2-8 °C, provided that they are handled carefully to avoid any environment contamination. Under these conditions the reagents are stable up to the expiry date stated on the labels.

AUXILIARY MATERIALS

- Semi automatic pipettes of 10, 200 and 1000 µl
- Vortex mixer and absorbent paper
- Chronometer
- Ultrapure Elisa grade water
- Microplate incubator at 37 ± 1 °C.
- Photometric reader of microplates or microstrips, linear up to at least 2 OD and supplied with filter of 450 nm (620- 630 nm).
- Automatic microplates washing device or manual apparatus capable of aspirating and dispensing volumes of 300 µl.

SAMPLES

Serum or plasma (heparin, EDTA)..Samples can be stored at 2–8 °C for a short time (max two days). For longer storage the specimen should be frozen. Avoid repeated freezing and thawing. Highly lipemic, hemolysed or microbiologically contaminated samples should not be used in the assay.

REAGENTS PREPARATION

- WASHING SOLUTION: dilute 1:10 with distilled or ELISA grade water (ex.: 20 ml of reagent C + 200 ml of distilled water) and mix carefully before use. The diluted washing solution can be stored for one week at room temperature or four weeks at +2-8°C. It is recommended to store diluted washing solution at room temperature for immediate use. In Reagent C it is possible to observe the presence of crystals, in this case mix at room temperature until complete dissolution of crystals.

WASHING INSTRUCTION

A good washing procedure is essential to obtain correct and precise analytical results.

We therefore recommend to use a good quality ELISA microplate washer, maintained at a good level of washing mechanical performances.

Generally, 2-3 automatic washing cycles of 0.3 ml/well are sufficient to avoid false positive reactions and remove high background. Anyhow we recommend to calibrate the washing system on the kit itself so to match the declared analytical performances.

In case of manual washing, we suggest to perform 3 washing cycles, dispensing and aspirating 0.3 ml/well per cycle.

In any case the liquid washed out from the plates must be inactivated with a sodium hypochlorite solution at a final concentration of 2.5%, before being thrown away or autoclaved, as it must be considered as potentially infected.

ASSAY PROCEDURE

1. At least one hour before use, bring all reagents, standards and samples to room temperature (18-30°C), mixing them carefully on vortex.
2. Do not mix reagents from different lots.
3. We recommend to distribute standards and samples in duplicate.
4. Distribution and incubation times must be the same for all wells in the same analysis.
5. Avoid long interruptions between each step of the assay procedure.
6. It is suggested to eliminate the excess of washing solution from the microplate after washing by blotting it gently on an absorbent paper pad.
7. The colour developed in the last incubation is stable for a maximum of one hour. Otherwise, in case of reading after 10-15 min after dispensing stop solution, immediately place the strips **in the dark**.
8. We recommend to read the plate with an ELISA automatic reader able to subtract the background at 620-630 nm and to read the absorbance of samples and standards at 450 nm. The "blanking" of the instrument is to be carried out in the blank reagent well (well A1).

ASSAY SCHEME

1. Put the desidered number of microstrips into the frame.
2. If suggested analyte concentration in the sample exceeds 2000 pg/ml, dilute this sample accordingly, using Standard 0.
3. Follow the scheme:

		Microplate wells coated with anti-Estradiol antibody		
	REAGENTS	**Blank**	**Standard**	**Sample**
Immunological reaction	Standard	-	25 µl	-
	Sample	-	-	25 µl
	Reagent B (Enzymatic Tracer)	-	200 µl	200 µl
	- Cover the strips with cardboard sealer - Incubate **120 minutes at 37 ± 1 °C**			
Washing	- Peel out the cardboard sealer and aspirate the reaction solution from all wells - Rinse 3 times with 300 µl of diluted washing solution, carefully aspirating off the remaining liquid			
Colorimetric reaction	Reagent D/E (Chromogen-Substrate)	100 µl	100 µl	100 µl
	- Cover the strips with cardboard sealer - Incubate **30 minutes at room temperature (22-28 °C)**, avoiding light exposure			
	Reagent F (Stop Solution)	100 µl	100 µl	100 µl
	Read the absorbance of each well against Blank at 450 nm (and 620-630 nm)			

QUALITY CONTROL

It is recommended, in each analytical run, to use control sera with known Estradiol values, to check the correspondence of the obtained results with those expected and consequently validate the data.

CALCULATION OF RESULTS

1. Calculate the mean of the absorbance (Em) for each point of the standard curve (S0 – S5) and of each sample.
2. Plot the mean value of absorbance of the standards (Em) against proper Estradiol concentrations. Draw the best-fit curve through the plotted points. (Ex.: Four Parameter Logistic).
3. Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in pg/ml.
4. If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the standards fall within 10% of the assigned concentrations.

EXPECTED VALUES

From data obtained by Minias Globe Diagnostics the following reference ranges are suggested. Otherwise, it is recommended that each laboratory establishes its own reference range.

Subjects	**Ranges (pg/ml)**
Children:	< 40
Male:	< 60
Female:	
follicular phase	30-100
ovulatory peak	130-350
luteal phase	50-180
menopause	< 60

Note:
The clinical significance of Estradiol determination can be invalidated if the patient was treated with cortisone or natural or synthetic steroids.

ANALYTICAL PERFORMANCES

Sensitivity

The lowest detectable concentration of Estradiol is 10 ng/ml at the 95% confidence limit.

Precision

a. Intra Assay Variation

Within run variation was determined by 16 replicate determination of two different control sera in the same analytical run. %CV values found were < 7% according to the optical density revealed.

b. Inter Assay Variation

Between run variation was determined by replicate measurements of three different control sera in 2 different lots. %CV values found were < 10.5% according to the optical density revealed.

Recovery

The recovery of 50 – 100 – 200 - 400 pg/ml of Estradiol added to sample gave an average value (± SD) of 98.4% ± 5.2%.

Correlation with RIA

The present kit was compared to a well-established RIA method. sera were assayed with both methods.
The following linear regression curve was calculated:

$y = 0.988x - 9.87$ pg/ml $r = 0.972$

Specificity

The cross reaction studied and relative results are shown in the following table:

Estradiol	100%
Estrone	2%
Estriol	0.4%
Testosterone	0.02 %
Cortisol	$< 7 \times 10^{-3}$ %
Progesterone	$< 3 \times 10^{-4}$ %
DHEA-S	$< 1 \times 10^{-4}$ %

"Hook" Effect

The method shows no "Hook" Effect up to 20.000 pg/ml.

PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentrations of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes. The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management

Please refer to local legal requirements.

REFERENCES

1. Joshi,U.M., Steroids 34 (1) 35 (1979)
2. D.Exley and R. Abuknesha Febs Letters 91,(2) 162 (1978)
3. Ismail A.A, et al J.Clin.Endocrin.Metab. 34,177-184 (1972)
4. Rajkowski,K.M, et al Steroids 29-5 (1977)
5. Wisdom G.B. Clin. Chem. 22/8, 1243-1255 (1976)
6. D. Sadem, et al J. of Immunolog. Methods 28 125-131(1979)

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