DRG® Estradiol ELISA (EIA-2693)

CE

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1 INTRODUCTION

1.1 Intended Use

The **DRG Estradiol ELISA** is an enzyme immunoassay for the quantitative *in vitro diagnostic* measurement of Estradiol in serum and plasma

1.2 Summary and Explanation

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 (1,2,3).

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 (4,5). 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 (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 luteinising hormone (LH), which are essential for folicular 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 feminising syndromes (14), gynaecomastia (15) and testicular tumors (16).

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

2 PRINCIPLE OF THE TEST

The DRG Estradiol ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding.
The microtiter wells are coated with a polyclonal [rabbit] antibody directed towards an antigenic site on the Estradiol molecule. Endogenous Estradiol of a patient sample competes with an Estradiol-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of Estradiol in the sample. After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of Estradiol in the patient sample.

3 WARNINGS AND PRECAUTIONS

- This kit is for in vitro diagnostic use only.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
- All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- Avoid contact with *Stop Solution* containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
- Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
  Material Safety Data Sheets for this product are available upon request directly from DRG.

4 REAGENTS

4.1 Reagents provided

1. ***Microtiterwells***, 12x8 (break apart) strips, 96 wells;
   Wells coated with a anti-Estradiol antibody (polyclonal).
2. ***Standard (Standard 0-6)*, 7 vials, 1 mL, ready to use;
   Concentrations: 0, 25; 100; 250; 500; 1000; 2000 pg/mL
   Conversion: 1 pg/mL = 3.67 pmol/L
   contain 0.03% Proclin 300 + 0.005% gentamicin sulfate as preservatives.
3. ***Enzyme Conjugate***, 1 vial, 25 mL, ready to use;
   Estradiol conjugated to horseradish peroxidase;
   * contains 0.03% Proclin 300, 0.015% BND and 0.010% MIT as preservatives.
4. ***Substrate Solution***, 1 vial, 14 mL, ready to use;
   Tetramethylbenzidine (TMB).
5. ***Stop Solution***, 1 vial, 14 mL, ready to use;
   contains 0.5M H_2SO_4.
   Avoid contact with the stop solution. It may cause skin irritations and burns.
6. ***Wash Solution***, 1 vial, 30 mL (40X concentrated);
   see „Preparation of Reagents".

* BND = 5-bromo-5-nitro-1,3-dioxane
MIT = 2-methyl-2H-isothiazol-3-one

Note: Additional *Standard 0* for sample dilution is available upon request.

4.2 Material required but not provided

- A microtiter plate calibrated reader (450 ± 10 nm), (e.g. the DRG Instruments Microtiter Plate Reader).
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or Deionized water
- Timer
- Semi-logarithmic graph paper or software for data reduction

4.3 Storage Conditions

When stored at 2°C - 8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
Opened reagents must be stored at 2°C - 8°C. Microtiter wells must be stored at 2°C - 8°C. Once the foil bag has been opened, care should be taken to close it tightly again.

4.4 Preparation of Reagents

Allow all reagents and required number of strips to reach room temperature prior to use.

Wash Solution

Add deionized water to the 40X concentrated Wash Solution.

Dilute 30 mL of concentrated *Wash Solution* with 1170 mL deionized water to a final volume of 1200 mL.

The diluted Wash Solution is stable for 2 weeks at room temperature.

4.5 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets (see chapter 13).

4.6 Damaged Test Kits

In case of any severe damage to the test kit or components, DRG has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5 SPECIMEN COLLECTION AND PREPARATION

Serum or plasma (EDTA-, Heparin- or citrate plasma) can be used in this assay.

Do not use haemolytic, icteric or lipaemic specimens.

Please note: Samples containing sodium azide should not be used in the assay.

5.1 Specimen Collection

Serum:

Collect blood by venipuncture (e.g. Sarstedt Monovette # 02.1388.001), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Plasma:

Whole blood should be collected into centrifuge tubes containing anti coagulant and centrifuged immediately after collection.

(E.g. for EDTA plasma Sarstedt Monovette – red cap - # 02.166.001;
for Heparin plasma Sarstedt Monovette – orange cap - # 02.165.001;
for Citrate plasma Sarstedt Monovette – green cap - # 02.167.001.)

5.2 Specimen Storage d Preparation

Specimens should be capped and may be stored for up to 5 days at 2°C - 8°C prior to assaying.

Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Standard 0* and reassayed 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 Serum + 90 µL *Standard 0* (mix thoroughly)
b) Dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Standard 0* (mix thoroughly).

6 ASSAY PROCEDURE

6.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

6.2 Test Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense **25 µL** of each ***Standard, Control*** and **samples** with new disposable tips into appropriate wells.
3. Dispense **200 µL** ***Enzyme Conjugate*** into each well.
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **120 minutes** at room temperature (without covering the plate).
5. Briskly shake out the contents of the wells.
   Rinse the wells **3 times** with diluted *Wash Solution* (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note:
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6. Add **100 µL** of ***Substrate Solution*** to each well.
7. Incubate for **15 minutes** at room temperature.
8. Stop the enzymatic reaction by adding **50 µL** of ***Stop Solution*** to each well.
9. Determine the absorbance (OD) of each well at **450±10 nm** with a microtiter plate reader.
   It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

6.3 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. 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.
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. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3.1 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.

Standard	Optical Units (450 nm)
Standard 0 (0 pg/mL)	2.40
Standard 1 (25 pg/mL)	1.92
Standard 2 (100 pg/mL)	1.25
Standard 3 (250 pg/mL)	0.72
Standard 4 (500 pg/mL)	0.48
Standard 5 (1000 pg/mL)	0.30
Standard 6 (2000 pg/mL)	0.21

7 EXPECTED VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted with apparently normal healthy adults, using the DRG Estradiol ELISA the following values are observed:

Population	**5 – 95% Percentile**
Males	10 - 36 pg/mL
Females	
pre-menopausal	13 - 191 pg/mL
post-menopausal	11 – 65 pg/mL

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

8 QUALITY CONTROL

Good laboratory practice requires that controls be 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.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor or DRG directly.

9 PERFORMANCE CHARACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between 9.7 pg/mL – 2000 pg/mL.

9.2 Specificity of Antibodies (Cross Reactivity)

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

Compound	% Cross reactivity		Compound	% Cross reactivity
Estradiol-17ß	100		11-Deoxycortisol	0
Androstenedione	0		21-Deoxycortisol	0
Androsterone	0		Dihydrotestosterone	0
Corticsterone	0		Dihydroepiandrosterone	0
Cortisone	0		20-Dihydroprogesterone	0
Epiandrosterone	0		11-Hydroxyprogesterone	0
16-Epiestriol	0		17α-Hydroxyprogesterone	0
Estradiol-3-sulfate	0		17α-Pregnenolone	0
Estradiol-3-glucoronide	0		17α-Progesterone	0
Estradiol-17α	0		Pregnanediol	0
Estriol	0.05		Pregnanetriol	0
Estriol-16-glucoronide	0		Pregnenolone	0
Estrone	0.2		Progesterone	0
Estrone-3-sulfate	0		Testosterone	0
Dehydroepiandrosterone	0			

9.3 Sensitivity

The analytical sensitivity was calculated from the mean minus two standard deviations of twenty (20) replicate analyses of *Standard 0* and was found to be 9.714 pg/mL.

9.4 Reproducibility

9.4.1 Intra Assay

The within assay variability is shown below:

Sample	n	Mean (pg/mL)	CV (%)
1	20	91.09	6.81
2	20	198.05	2.71
3	20	307,71	4,13

9.4.2 Inter Assay

The between assay variability is shown below:

Sample	n	Mean (pg/mL)	CV (%)
1	12	90.00	7.25
2	12	197.21	6.72
3	12	299.74	9.39

9.5 Recovery

Samples have been spiked by adding Estradiol solutions with known concentrations in a 1:1 ratio.
The expected values were calculated by addition of half of the values determined for the undiluted samples and half of the values of the known solutions. The % Recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100.

Sample	Added Concentration 1:1 (v/v) (pg/mL)	Measured Conc. (pg/mL)	Expected Conc. (pg/mL)	Recovery (%)
1		63.51		
	2000	1009.69	1031.76	97.9
	1000	537.45	531.76	101.1
	500	268.78	281.76	95.4
	250	135.39	156.76	86.4
2		161.26		
	2000	1171.98	1080.63	108.5
	1000	622.81	580.63	107.3
	500	361.57	330.63	109.4
	250	188.77	205.63	91.8
3		285.32		
	2000	1072.26	1142.66	93.8
	1000	579.67	642.66	90.2
	500	359.47	392.66	91.5
	250	241.75	267.66	90.3

9.6 Linearity

Sample	Dilution	Mean Conc. (pg/mL)	Recovery (%)
1	None	63.51	
	1:2	31.10	97.9
	1:4	16.84	106.1
	1:8	8.90	112.1
	1:16	3.74	94.2
2	None	161.21	
	1:2	85.38	105.9
	1:4	42.22	104.8
	1:8	17.80	88.3
	1:16	11.01	109.3
3	None	285.32	
	1:2	140.01	98.1
	1:4	67.59	94.8
	1:8	35.52	99.6
	1:16	16.80	94.2

10 LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.
Any improper handling of samples or modification of this test might influence the results.

10.1 Interfering Substances

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results.

10.2 Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of Estradiol in a sample.

10.3 High-Dose-Hook Effect

No hook effect was observed in this test.

11 LEGAL ASPECTS

11.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact DRG.

11.2 Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

11.3 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid.
Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

12 REFERENCES / LITERATURE

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