DRG® Estradiol CLIA (CLA-4664)

CE

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IVD

1 INTRODUCTION

1.1 Intended Use

The **DRG Estradiol CLIA** is a chemiluminescence 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; E21) 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, 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).
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 fertilization (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 CLIA is a **c**hemi**l**uminescence **i**mmuno**a**ssay (CLIA), based on the principle of competitive binding.
The microtiter wells are coated with a polyclonal antibody [rabbit] directed towards a unique 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 reverse proportional to the concentration of Estradiol in the sample. After addition of the substrate solution, the intensity of emitted light is inversely proportional to the concentration of Estradiol in the patient sample.

3 PRECAUTIONS

1. This kit is for in vitro diagnostic use only.
2. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
3. 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.
4. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
5. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
6. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
7. Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
8. Do not use reagents beyond expiry date as shown on the kit labels.
9. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate luminometer.
10. The luminescence substrate reagents (*Reagent A* and *Reagent B*) are sensitive to light and should be stored in the original dark bottle away from direct sunlight.
11. 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.
12. Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.
13. Safety Data Sheets for this product are available upon request directly from DRG International, Inc.
    The Safety Data Sheets fit the demands of: EU-Guideline 91/155 EC.

4 KIT COMPONENTS

4.1 Contents of the Kit

1. ***Microtiterwells***, 12x8 (break apart) strips, 96 wells;
   Wells coated with a anti-Estradiol antibody (polyclonal rabbit antibody).
2. ***Standard (Standard 0-5*)**, 6 vials, 1 mL, ready to use
   Concentration: 0 – 25 – 100 – 250 – 500 - 1000 pg/mL
   Conversion: 1 pg/mL = 3.67 pmol/L
   contain 0.03% Proclin 300 and 0.005% gentamicin sulfate as preservatives.
3. ***Enzyme Conjugate***, 1 vial, 12 mL, ready to use;
   Estradiol conjugated to horseradish Peroxidase.
   * contains 0.03% Proclin 300, 0.015% BND and 0.010% MIT as preservatives.
4. ***Chemiluminescence Substrate Solution***,
   Reagent A, 1 vial, 2 mL, *Note: light sensitive!*
   Reagent B, 1 vial, 2 mL, *Note: light sensitive!*
   Reagent C, 1 vial, 3 mL
   see „*Preparation of Reagents*“.
5. ***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.

4.1.1 Equipment and material required but not provided

- A microtiter plate luminometer.
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Aqua dest.

4.2 Storage and stability of the Kit

When stored at 2°C-8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
All opened reagents must be stored at 2°C-8°C. Microtiter wells must be stored at 2°C-8°C. Once the foilbag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for two months if stored as described above.

4.3 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.

Chemiluminescence Substrate Solution

Mix **1 part** of the chemiluminescence ***Reagent A*** with **1 part** of ***Reagent B*** and dilute this mixture 1:2 with ***Reagent C***.
This gives the ready to use substrate solution.
The prepared substrate solution is stable for one hour. Prepare fresh before use.
If the whole plate is to be used prepare the substrate solution as follows:
Add 1.5 mL of each *Reagent A* and *Reagent B* into 3 mL *Reagent C*.

4.4 Disposal of the Kit

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

4.5 Damaged Test Kits

In case of any severe damage of the test kit or components, DRG have to be informed written, 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

Serum or plasma (EDTA-, Heparin- or citrat 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.

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 Citrat plasma Sarstedt Monovette – green cap - # 02.167.001.)

5.2 Specimen Storage

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 serum specimen is found to contain more than the highest standard, the specimens can be diluted 10-fold or 100 fold 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 TEST 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
- Light intensity 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 Assay Procedure

Each run must include a standard curve.

1. Secure the desired number of *Microtiterwells* in the holder.
2. Dispense **20 µL** of each *Standard*, controls and samples with new disposable tips into appropriate wells.
3. Dispense **100 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 **35 minutes** at room temperature without covering the plate.
5. Briskly shake out the contents of the wells.
   Rinse the wells **5 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 **50 µL** of the freshly prepared Substrate Solution to each well. *(See "Preparation of Reagents.)*
7. Incubate for **10 minutes** at room temperature.
8. Read the RLU with a microtiter plate luminometer **within 20 minutes** after incubation time of substrate.

6.3 Calculation of Results

1. Calculate the average Relative Light Units (RLU) values for each set of standards, controls and patient samples.
2. Construct a standard curve by plotting the mean RLU obtained from each standard against its concentration with RLU value on the vertical(Y) axis and concentration on the horizontal (X) axis.
3. Using the mean RLU 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.

Standards (pg/mL)	RLU (x10^3)	RLU/RLU_{max} (%)
Standard 0 (0)	1607	100
Standard 1 (25)	1030	64.1
Standard 2 (100)	379	23.6
Standard 3 (250)	137	8.5
Standard 4 (500)	50	3.1
Standard 5 (1000)	21	1.3

** It is recommended to use the RLU/RLU_{max} values for comparative purposes since luminometers vary considerably between manufacturers. Results from different luminometers will show different RLU values, however, the RLU/RLU_{max} values remain consistent.

7 EXPECTED VALUES

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

The following values can be used as a guideline:

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

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 ASSAY CHARACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between 0 – 1000 pg/mL.

9.2 Specificity of Antibodies (Cross Reactivity)

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

Compound	% Cross reactivity
Estradiol-17β	100
Estriol	1.84
Androstenedione	0.08
Corticosterone	0.001
Cortisone	0
Estrone	0
Progesterone	0
Testosterone	0
Dehydroepiandrosterone	0.005
Dehydroepiandrosterone Sulfate	0
17α-Hydroxyprogesterone	0.005
Prednisone	0
Cortisol	0.001

9.3 Analytical 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 2.5 pg/mL.

9.4 Precision

9.4.1 Intra Assay Variation

The intra assay (within-run) variability is shown below:

Sample	Serum 1	Serum 2	Serum 3
Mean [pg/mL]	115.94	63.37	298.81
CV (%)	4.35	4.49	4.58
n =	20	20	20

9.4.2 Inter Assay Variation

The inter assay (between-run) variability is shown below:

Sample	Serum 1	Serum 2	Serum 3
Mean [pg/mL]	37.56	11.93	42.04
CV (%)	5.66	8.62	5.10
n =	18	18	12

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 1	Sample 2	Sample 3
Concentration [pg/mL]		11.07	2.59	81.76
Average Recovery [%]		96.3	93.6	92.9
Range of Recovery [%]	from	90.6	88.3	87.9
	to	99.2	100.6	101.7

9.6 Linearity

		Sample 1	Sample 2	Sample 3
Concentration [pg/mL]		136.26	114.04	163.26
Average Recovery [%]		100.6	102.5	100.7
Range of Recovery [%]	from	92.1	86.6	93.3
	to	108.7	113.6	111.3

10 LIMITATIONS OF USE

10.1 Interfering Substances

Any improper handling of samples or modification of this test might influence the results.

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

9. Tsang, B.K., Armstrong, D.T. and Whitfield, J.F., Steroid biosyntheses by isolated human ovarian follicular cells in vitro, J. Clin. Endocrinol. Metab. 51:1407 - 11 (1980).
10. Gore-Langton, R.E. and Armstrong, D.T., Follicular stoidogenesis and its control. In: The physiology of Reproduction, Ed.: Knobil, E., and Neill, J. et al., pp. 331-85. Raven Press, New York (1988).
11. Hall, P.F., Testicular Steroid Synthesis: Organization and Regulation. In: The Physiology of Reproduction, Ed.: Knobil, E., and Neill, J. et al., pp 975-98. Raven Press, New York (1988).
12. Siiteri, P.K. Murai, J.T., Hammond, G.L., Nisker, J.A., Raymoure, W.J. and Kuhn, R.W., The serum transport of steroid hormones, Rec. Prog. Horm. Res. 38:457 - 510 (1982).
13. Martin, B., Rotten, D., Jolivet, A. and Gautray, J-P-. Binding of steroids by proteins in follicular fluid of the human ovary, J.Clin. Endicrinol. Metab. 35: 443-47 (1981).
14. Baird, D.T., Ovarian steroid secretion and metabolism in women. In: The Endocrine Function of the Human Ovary. Eds.: James, V.H:T., Serio, M. and Giusti, G. pp. 125-33, Academic Press, New York (1976).
15. McNastty, K.P., Baird, D.T., Bolton, a., Chambers, P., Corker, C.S. and McLean, H., concentration of oestrogens and androgens in human ovarian venous plasma and follicular fluid throughout the menstrual cycle, J. Endocrinol. 71:77-85 (1976).
16. Abraham, G.E., Odell, W.D., Swerdloff, R.S., and Hopper, K., Simultaneous radioimmunoassay of plasma FSH, LH, progesterone, 17-hydroxyprogesterone and estradiol-17ß during the menstrual cycle, J.Clin. Endocrinol. Metab. 34:312-18 (1972).
17. March, C.M., Goebelsmann, U., Nakumara, R.M., and Mishell, D.R., Roles of oestradiol and progesterone in eliciting midcycle luteinising hormone and follicle stimulating hormone surges. J. clin. Endicrinol. Metab. 49:507-12 (1979).
18. Simpson, E.R., and McDonald, P.C., Endocrinology of Pregnancy. In: Textbook of Endocrinology, Ed.: Williams, R.H. pp412-22, Saunders Company, Philadelphia (1981).
19. Jenner, M.R., Kelch, R.P., et al., Hormonal Changes in prepubertal children, pubertal females and in precocious puberty, premature thelarche, hypogonadism and in a child with feminising tumour, J. clin. Endocrinol. 34: 521 (1982).
20. Goldstein, D. et al., Correlation between oestradiol and progesterone in cycles with luteal phase deficiency, Fertil. Steril. 37: 348-54 (1982).
21. Kirschner, M.A., therole of hormones in the etiology of human breast cancer, Cancer 39:2716 26 (1977).
22. Odell, W.D. and Swerdloff, R.D., Abnormalities of gonadal function in men, clin. Endocr. 8:149-80 (1978).
23. McDonald, P.c., Madden, J.C., Brenner, P.F., Wilson, J.D. and Siiteri, P.K. Origin of oestrogen in normal men and women with testicular feminisation, J.Clin. Endcrinol. Metabol. 49:905 (1979).
24. Peckham, M.J: and McElwain, T.J:, Testicular tumours, J.Clin. Endocrinol. Metab. 4:665-692 (1975).
25. Taubert, H.d. and Dericks-Tan, J.s.E., Induction ofr ovulation by clomiphene citrate in combination with high doses of oestrogens or nasal application of LH-RH. In: Ovulation in the Human. Eds.: Crosignandi, P.G. and Mishell, D.R., pp.265-73, Academic Press, New York (1976).
26. Fishel, S.B., Edwards, R.G., Purdy, J.M., Steptoe, P.C., Webster, J. Walters, E., cohen, J. Fehilly, C. Hewitt, J., and Rowland, G., Implantation, abortion and birth after in-vitro fertilisation using the natural menstrual cycle or follicular stimulation with clomiphene citrate and human menopausal gonadotropin, J. In Vitro Fertil. Embryo Transfer, 1:24-28 (1985).

27. Wramsby, H., Sundstorm, P- and Leidholm, P., Pregnancy rate in relation to number of cleaved eggs replaced after in vitro-fertilisation of stimulating cycles monitored by serum levels of oestradiol and progesterone as sole index. Human Reproduction 2: 325-28 (1987).
28. Ratcliff, W.A.., Carter, G.D., et al., Estradiol assays: applications and guidelines for the provision of clinical biochemistry service, Ann. Clin. Biochem. 25:466-483 (1988).
29. Tietz, N.W. Textbook of Clinical Chemistry. Saunders, 1986.