



User's Manual

# Estradiol ELISA

Enzyme immunoassay for the in-vitro diagnostic quantitative determination of Estradiol in human serum and plasma

**IVD**

**REF**

**IB79331**



**96 Wells**

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## 1 INTRODUCTION

### 1.1 Intended Use

The **IBL-AMERICA Estradiol ELISA** is a competitive immunoassay for the quantitative measurement of estradiol in serum and plasma (EDTA).

### 1.2 Summary and explanation

Estradiol (1,3,5(10)-estratriene-3,17 $\beta$ -diol; 17 $\beta$ -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, 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 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 fertilisation (IVF), serum estradiol concentrations are usually monitored daily for optimal timing of human chorionic gonadotropin (hCG) administration and oocyte collection (19).

## 2 PRINCIPLE

The **IBL-AMERICA 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 an anti-Estradiol antibody. An unknown amount of estradiol present in the 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 color developed is inversely proportional to the concentration of Estradiol in the sample.

### 3 WARNINGS AND PRECAUTIONS

1. This kit is for in vitro use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
4. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
5. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
6. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
7. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
8. Allow the reagents to reach room temperature (21-26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
9. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
10. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
11. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
12. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
13. Do not use reagents beyond expiry date as shown on the kit labels.
14. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
15. 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.
16. Avoid contact with Stop Solution. It may cause skin irritation.
17. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
18. For information please refer to Material Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from IBL-America.

## 4 REAGENTS

### 4.1 Reagents provided

1. **SORB MT Microtiterplate**, 12 x 8 (break apart) strips with 96 wells;  
Wells coated with anti-Estradiol antibody.
2. **CAL 0-5 Calibrators (Calibrator 0-5)**, 6 vials, 0.3 ml each, ready to use;  
Concentrations: 0 – 25 – 75 – 225 – 675 – 2000 pg/ml
3. **CONTROL 1-2 Control low / Control high**, 2 vials, 0.3 ml each, ready to use;  
containing Estradiol in human serum-buffer matrix.  
For control values and ranges please refer to QC-Datasheet
4. **ENZ CONJ Enzyme Conjugate**, 1 vial, 11 ml, ready to use;  
17 $\beta$ -Estradiol labeled horseradish peroxidase in buffered matrix.
5. **SUB TMB Substrate Solution**, 1 vial, 22 ml, ready to use;  
contains tetramethylbenzidine (TMB).
6. **STOP SOLN Stop Solution**, 1 vial, 7 ml, ready to use;  
contains 2 N hydrochloric acid solution.
7. **WASH SOLN 10x Wash Solution**, 1 vial, 50 ml (10X concentrated);  
see "Reagent Preparation".

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

### 4.2 Materials required but not provided

- A microtiter plate reader capable for endpoint measurement at 450 nm
- Calibrated variable precision micropipettes (25  $\mu$ l, 100  $\mu$ l, 200  $\mu$ l, 300  $\mu$ l).
- Microplate mixer operating at more than 600 rpm
- 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 to 8°C unopened reagents will be stable until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2°C to 8°C for 30 days. Microtiter wells must be stored at 2°C to 8°C. Take care that the foil bag is sealed tightly.

### 4.4 Reagent preparation

Allow the reagents and the required number of wells to reach room temperature (21-26°C) before starting the test.

#### Wash Solution:

Dilute 50 ml of 10X concentrated *Wash Solution* with 450 ml deionized water to a final volume of 500 ml. *The diluted Wash Solution is stable for at least 3 months at room temperature (21-26°C).*

### 4.5 Disposal of the kits

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 Sheet.

### 4.6 Damaged test kits

In case of any severe damage of the test kit or components, IBL-AMERICA 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

For determination of estradiol **serum or plasma (EDTA)** can be used. The procedure calls for 25 µl sample per well. The samples should be assayed immediately or aliquoted and stored at ≤ -20°C. Avoid repeated freeze-thaw cycles. Samples expected to contain estradiol concentrations higher than the highest calibrator (2000 pg/ml) should be diluted with the zero calibrator before assay. The additional dilution step has to be taken into account for the calculation of the results. Do not use grossly haemolytic, icteric or grossly lipaemic specimens.

## 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.
- Respect the incubation times as stated in this instructions for use.

### 6.2 Assay procedure

Each run must include a standard curve.

1. Prepare a sufficient number of microplate wells to accommodate calibrators and samples in duplicates.
2. Dispense **25 µl** of each **Calibrator, Sample and Control** with new disposable tips into appropriate wells.
3. Incubate for **60 minutes** at room temperature on a plate shaker (> 600 rpm).
4. Add **100 µl of Enzyme Conjugate** into each well.
5. Incubate for **60 minutes** at room temperature on a plate shaker (> 600 rpm)

**Important Note:**

Optimal reaction in this assay is markedly dependent on shaking of the microplate!

6. Discard the content of the wells and rinse the wells **4 times** with diluted **Wash Solution** (300 µl per well). Remove as much Wash Solution as possible by beating the microplate on absorbent paper.
7. Add **200 µl of Substrate Solution** to each well.
8. Incubate without shaking for **30 minutes** in the dark.
9. Stop the reaction by adding **50 µl of Stop Solution** to each well.
10. Determine the absorbance of each well at 450 nm. It is recommended to read the wells within 15 minutes.

**6.3 Calculation of results**

1. Calculate the average absorbance values for each set of calibrators, controls and samples.
2. Using semi logarithmic graph paper, 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 and control, determine the corresponding concentration from the calibration curve.
4. Automated method: The results in the package insert have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred calculation method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be determined directly from this calibrator curve. Samples with concentrations higher than that of the highest calibrator have to be further diluted. For the calculation of the concentrations, this dilution factor has to be taken into account.

**Example of typical calibrator curve**

Following data are intended for illustration only and should not be used to calculate results from another run.

Calibrator	Optical Units (450nm)
Calibrator 0 (0 pg/ml)	2.738
Calibrator 1 (25 pg/ml)	2.439
Calibrator 2 (75 pg/ml)	1.999
Calibrator 3 (225 pg/ml)	1.320
Calibrator 4 (675 pg/ml)	0.711
Calibrator 5 (2000 pg/ml)	0.346

**7 EXPECTED VALUES**

It is strongly recommended that each laboratory should establish its own normal ranges.

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

Population	Age	5%-95% Percentile
<b>Males</b>	< 50 years	n.d. – 36.2 pg/ml
	> 50 years	n.d. – 51.1 pg/ml
<b>Females</b>	< 50 years	n.d. – 111.4 pg/ml
	> 50 years	n.d. – 37.9 pg/ml

n.d.= non detectable

The values provided here were determined without consideration of the phase of menstrual cycle. 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 PERFORMANCE CHARACTERISTICS

### 8.1 Analytical Sensitivity

The lowest analytical detectable level of Estradiol that can be distinguished from the Zero Calibrator is 6.2 pg/ml at the 2SD confidence limit.

### 8.2 Specificity (Cross Reactivity)

The following materials have been evaluated for cross reactivity. The percentage indicates cross reactivity at 50% displacement compared to Estradiol.

Steroid	% Cross reaction
Androstenedione	< 0.1
17-Hydroxyprogesterone	< 0.1
Corticosterone	< 0.1
Estriol	0.4
Estrone	4.2
Pregnenolone	< 0.1
E2-3-Glucuronide	3.8
E2-3-Sulphate	3.6
E2-17-Glucuronide	< 0.1
Progesterone	< 0.1
Testosterone	< 0.1
Fulvestrant	9.5

### 8.3 Assay dynamic range

The range of the assay is between 25 – 2000 pg/ml.

### 8.4 Reproducibility

#### 8.4.1 Intra-Assay

The intra-assay variation was determined by 20 replicate measurements of three serum samples within one run. The within-assay variability is shown below:

	Serum 1	Serum 2	Serum 3
<b>Mean (pg/ml)</b>	83.8	269.9	794.8
<b>SD</b>	5.3	8.5	29.5
<b>CV (%)</b>	6.4	3.1	3.7
<b>n =</b>	20	20	20

#### 8.4.2 Inter-Assay

The inter-assay (between-run) variation was determined by duplicate measurements of three serum samples in 10 different tests.

	Serum 1	Serum 2	Serum 3
<b>Mean (pg/ml)</b>	85.2	251.3	727.8
<b>SD</b>	4.7	11.7	32.6
<b>CV (%)</b>	5.5	4.7	4.5
<b>n =</b>	10	10	10



## 8.5 Recovery

Using the calibrator matrix two spiking solutions of 100 ng/ml and 10 ng/ml were prepared. Three sera (1-3) were spiked with 10 and 25 µl of the 10 ng/ml solution and with 6 µl of the 100 ng/ml solution leaving the serum matrices relatively intact. All samples were measured by the IBL-AMERICA Estradiol ELISA procedure.

Sample	Spiking (pg/ml)	Measured (pg/ml)	Expected (pg/ml)	Recovery (%)
1	-	27.8	-	-
	100	141.3	127.8	111%
	250	315.0	277.8	113%
	600	685.0	627.8	109%
2	-	10.3	-	-
	100	119.3	110.3	108%
	250	267.4	260.3	103%
	600	710.3	610.3	116%
3	-	4.2	-	-
	100	98.7	104.2	95%
	250	267.3	254.2	105%
	600	668.1	604.2	111%

## 8.6 Linearity

Three serum samples were assayed undiluted and diluted with the zero calibrator.

Serum	Dilution	Measured (pg/ml)	Expected (pg/ml)	Linearity (%)
1	-	715.9	./.	./.
	1 in 2	321.3	358.0	90%
	1 in 4	164.4	179.0	92%
	1 in 8	88.9	89.5	99%
2	-	1012.7	./.	./.
	1 in 2	553.3	506.4	109%
	1 in 4	306.0	253.2	121%
	1 in 8	146.8	126.6	116%
3	-	1099.2	./.	./.
	1 in 2	583.4	549.6	106%
	1 in 4	316.1	274.8	115%
	1 in 8	165.0	137.4	120%

## 9 LIMITATIONS OF PROCEDURE

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.

### Drug Interferences

Any medication (cream, oil, pill etc.) containing Estradiol will significantly influence the measurement of this analyte.

The Estradiol ELISA should not be used for patients being treated with the drug fulvestrant (Faslodex®) which cross reacts in the Estradiol ELISA and could lead to falsely elevated test results.

## 10 LEGAL ASPECTS

### 10.1 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 IBL-AMERICA.

### 10.2 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 10.1. 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.

### 10.3 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 10.2. 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.












#### **Manufactured for:**

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**11 REFERENCES**

1. 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).
2. Gore-Langton, R.E. and Armstrong, D.T., Follicular steroidogenesis and its control. In: The physiology of Reproduction, Ed.: Knobil, E., and Neill, J. et al., pp. 331-85. *Raven Press*, New York (1988).
3. 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).
4. 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).
5. Martin, B., Rotten, D., Jolivet, A. and Gautray, J-P., Binding of steroids by proteins in follicular fluid of the human ovary, *J.Clin. Endocrinol. Metab.* 35: 443-47 (1981).
6. 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).
7. 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).
8. Abraham, G.E., Odell, W.D., Swerdloff, R.S. and Hopper, K., Simultaneous radioimmunoassay of plasma FSH, LH, progesterone, 17-hydroxyprogesterone and estradiol-17 $\beta$  during the menstrual cycle, *J.Clin. Endocrinol. Metab.* 34:312-18 (1972).
9. 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. Endocrinol. Metab.* 49:507-12 (1979).
10. Simpson, E.R. and McDonald, P.C., Endocrinology of Pregnancy. In: Textbook of Endocrinology, Ed.: Williams, R.H. pp412-22, *Saunders Company*, Philadelphia (1981).
11. 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).
12. Goldstein, D. et al., Correlation between oestradiol and progesterone in cycles with luteal phase deficiency, *Fertil. Steril.* 37: 348-54 (1982).
13. Kirschner, M.A., The role of hormones in the etiology of human breast cancer, *Cancer* 39:2716 26 (1977).
14. Odell, W.D. and Swerdloff, R.D., Abnormalities of gonadal function in men, *Clin. Endocr.* 8:149-80 (1978).
15. 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. Endocrinol. Metabol.* 49:905 (1979).
16. Peckham, M.J: and McElwain, T.J., Testicular tumours, *J.Clin. Endocrinol. Metab.* 4:665-692 (1975).
17. Taubert, H.D. and Dericks-Tan, J.S.E., Induction of 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).
18. 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).
19. 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).
20. 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).
21. Tietz, N.W., *Textbook of Clinical Chemistry*. Saunders, 1986.

**SYMBOLS USED WITH IBL-AMERICA ELISA**

Symbol	English	Deutsch	Français	Espanol	Italiano
	European Conformity	CE-Konfirmitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
<i>Distributed by</i>	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore