Biogradetech

Product information

Estradiol ELISA

BGT-KET-512

96 Tests

Please use only the valid version of the Instructions for Use provided with the kit.

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1 INTRODUCTION

1.1 Intended Use

The **Estradiol ELISA** is an enzyme immunoassay for the quantitative in vitro diagnostic measurement of estradiol in serum or plasma (EDTA, lithium heparin or citrate 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, 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 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 of the estradiol molecule. During the first incubation, the estradiol in the added sample competes with the added enzyme conjugate , which is estradiol conjugated to horseradish peroxidase , for binding to the coated antibody . After a washing step to remove all unbound substances, the solid phase is incubated with the substrate solution. The colorimetric reaction is stopped by addition of stop solution, and optical density (OD) of the resulting yellow product is measured. The intensity of colour is inversely proportional to the concentration of the analyte in the sample. A standard curve is constructed by plotting OD values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

3 WARNINGS AND PRECAUTIONS

1. This kit is for in vitro diagnostic use only. For professional use only.
2. 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.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of instructions for use provided with the kit. Be sure that everything is understood.
4. 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.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. 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 coloured. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (20 °C to 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
16. 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.
17. Avoid contact with Stop Solution containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Safety Data Sheets.

4 REAGENTS

4.1 Reagents provided

1. **SORB MT Microtiterwells**, 12 x 8 (break apart) strips, 96 wells; Wells coated with anti-estradiol antibody (polyclonal).
2. **CAL 0 – 6 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 non-mercury preservative.
3. **CONTROL low & high Control Low & High**, 2 vials, 1 mL, ready to use; For control values and ranges please refer to Certificate of Analysis. Contain non-mercury preservative
4. **ENZ CONJ Enzyme Conjugate**, 1 vial, 14 mL, ready to use, estradiol conjugated to horseradish peroxidase; Contains non-mercury preservative.
5. **SUB TMB Substrate Solution**, 1 vial, 14 mL, ready to use, Tetramethylbenzidine (TMB).
6. **STOP SOLN Stop Solution**, 1 vial, 14 mL, ready to use, contains 0.5 M H_2SO_4,
   Avoid contact with the stop solution. It may cause skin irritations and burns.
7. **WASH SOLN 40x Wash Solution**, 1 vial, 30 mL (40X concentrated),
   See “Reagent Preparation“.

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

4.2 Materials required but not provided

- A calibrated microtiter plate reader (450 nm, with reference wavelength at 620 nm to 630 nm)
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled 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 retain reactivity until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again. Opened kits retain activity for 8 weeks if stored as described above.

4.4 Reagent Preparation

Bring all reagents and required number of strips to room temperature (20 °C to 26 °C) prior to use.

Wash Solution

Add deionized water to the 40X concentrated Wash Solution. Dilute 30 mL of concentrated Wash Solution with 1170 mL distilled water to a final volume of 1200 mL. The diluted Wash Solution is stable for 1 week at room temperature.

4.5 Disposal of the Kit

The disposal of the kit and all used materials/reagents must be performed according to the national regulations. Special information for this product is given in the Safety Data Sheet, section 13.

4.6 Damaged Test Kits

In case of any severe damage to the test kit or components , It 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, lithium heparin or citrate plasma) can be used in this assay.
Note: Samples containing sodium azide should not be used in the assay. In general, it should be avoided to use haemolytic, icteric, or lipaemic specimens. For further information refer to chapter "Interfering Substances".

5.1 Specimen Collection

Serum:
Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), 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 (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

5.2 Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 7 days at 2 °C to 8 °C prior to assaying.
Specimens held for a longer time (up to one year) 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 sample + 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.
- Optical density 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 **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 **90 minutes** at room temperature.
5. Rinse the wells **3 times** with 400 µL diluted Wash Solution per well, if a plate washer is used
   - OR-
   Briskly shake out the contents of the wells.
   Rinse the wells **3 times** with 400 µL diluted Wash Solution per well for manual washing. 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 **30 minutes** at room temperature.
8. Stop the enzymatic reaction by adding **50 µL** of **Stop Solution** to each well.
9. Determine the optical density of the solution in each well at 450 nm (reading) and at 620 nm to 630 nm (background subtraction, recommended) 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 optical density (OD) values for each set of standards, controls and patient samples.
2. Using semi-logarithmic graph paper, construct a standard curve by plotting the mean OD obtained from each standard against its concentration with OD value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean OD value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the Instructions for Use have been calculated automatically using a 4-Parameter curve fit. (4 Parameter Rodbard or 4 Parameter Marquardt are the preferred methods.) 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 or reported as > 2000 pg/mL. 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 Density (450 nm)
Standard 0 (0 pg/mL)	2.09
Standard 1 (25 pg/mL)	1.83
Standard 2 (100 pg/mL)	1.49
Standard 3 (250 pg/mL)	1.15
Standard 4 (500 pg/mL)	0.85
Standard 5 (1000 pg/mL)	0.55
Standard 6 (2000 pg/mL)	0.28

7 EXPECTED NORMAL 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 Estradiol ELISA the following values are observed:

Population	n	Range (pg/mL)	Mean (pg/mL)	Median (pg/mL)	2.5^{th} – 97.5^{th} Percentile (pg/mL)
Males	30	30.1 – 68.1	50.4	51.2	30.0 – 68.0
Females					
pre-menopausal					
follicular phase	40	28.1 – 178.1	82.4	75.0	28.3 – 173.2
ovulation	25	51.2 – 549.0	166.4	132.7	53.5 – 465.6
luteal phase	36	33.6 – 250.9	93.9	89.3	38.7 – 172.4
post-menopausal	14	18.4 – 64.0	37.6	37.5	19.0 – 63.2

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 directly.

9 PERFORMANCE CHARACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between 10.6 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	0.003
Estradiol-3-sulfate	0		17α-Pregnenolone	0
Estradiol-3-glucoronide	0		17α-Progesterone	0
Estradiol-17α	0		Pregnanediol	0
Estriol	2.27		Pregnanetriol	0
Estriol-16-glucoronide	0		Pregnenolone	0
Estrone	6.86		Progesterone	0
Estrone-3-sulfate	0		Testosterone	0.033
Dehydroepiandrosterone	0		Fulvestrant	3.7

9.3 Sensitivity

The analytical sensitivity of the ELISA was calculated by subtracting 2 standard deviations from the mean of 20 replicate analyses of the Standard 0 and was found to be 10.6 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	92.5	9.2
2	20	144.4	9.0
3	20	340.7	8.7

9.4.2 Inter Assay

The between assay variability is shown below:

Sample	n	Mean (pg/mL)	CV (%)
1	40	151.3	14.9
2	40	336.7	10.8
3	39	661.4	6.9

9.4.3 Inter-Lot

The inter-assay (between-lots) variation was determined by measuring each sample 6 times with 2 different kit lots:

Sample	n	Mean (pg/mL)	CV (%)
1	12	309.0	10.1
2	12	475.1	11.2
3	12	681.4	11.7

9.5 Recovery

Samples have been spiked by adding estradiol solutions with known concentrations.
The % recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100

		Serum 1	Serum 2	Serum 3	EDTA plasma	Heparin plasma	Citrate plasma
Concentration (pg/mL)		254.0	456.0	745.9	168.0	225.2	203.1
Average Recovery (%)		97.3	93.3	102.0	101.8	98.1	96.5
Range of Recovery (%)	from	85.6	87.0	97.0	95.7	93.2	91.3
	to	112.2	108.0	112.5	112.4	108.9	104.8

9.6 Linearity

Samples were measured undiluted and in serial dilutions with standard 0. The recovery (%) was calculated by multiplying the ratio of expected and measured values with 100.

		Serum 1	Serum 2	Serum 3	EDTA plasma	Heparin plasma	Citrate plasma
Concentration (pg/mL)		337.4	456.0	650.2	892.5	979.6	857.0
Average Recovery (%)		98.1	101.1	105.4	92.5	91.4	93.5
Range of Recovery (%)	from	90.9	85.1	101.0	85.2	87.8	86.0
	to	101.5	108.8	114.4	111.0	95.8	102.2

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 7.5 mg/mL) have no influence on the assay results.

10.2 Drug Interferences

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.

10.3 High-Dose-Hook Effect

A High-Dose-Hook Effect is not known for competitive assays.

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.

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.