

## REFERENCES

1. Tsang, B.K., Armstrong, D.T. and Whitfield, J.F., Steroid biosynthesis by isolated human ovarian follicular cells in vitro, J. Clin. Endocrinol. Metab., 1980; 51: 1407-1411.
2. Gore-Langton, R.E. and Armstrong, D.T., Follicular steroidogenesis and its control. In: Knobil, E., and Neill, J. et al., ed. The Physiology of Reproduction. Raven Press, New York; 1988: 331-385.
3. Hall, P.F., Testicular steroid synthesis: Organization and regulation. In: Knobil, E., and Neill, J. et al., ed. The Physiology of Reproduction. Raven Press, New York; 1988: 975-998.
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., 1982; 38: 457-510.
5. Baird, D.T., Ovarian steroid secretion and metabolism in women. In: James, V.H.T., Serio, M. and Giusti, G., eds. The Endocrine Function of the Human Ovary. Academic Press, New York; 1976: 125-133.
6. McNatty, 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., 1976; 71: 77-85.
7. 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., 1972; 34: 312-318.
8. March, C.M., Goebelsmann, U., Nakumara, R.M., and Mishell, D.R. Jr., Roles of estradiol and progesterone in eliciting the midcycle luteinizing hormone and follicle-stimulating hormone surges. J. Clin. Endocrinol. Metab., 1979; 49: 507-513.
9. Simpson, E.R., and MacDonald, P.C., Endocrinology of pregnancy. In: Williams, R.H., ed., Textbook of Endocrinology. Saunders Company, Philadelphia; 1981: 412-422.
10. Jenner, M.R., Kelch, R.P., Kaplan, S.L. and Grumbach, M.M., Hormonal changes in puberty: IV. Plasma estradiol, LH, and FSH in prepubertal children, pubertal females and in precocious puberty, premature thelarche, hypogonadism and in a child with feminizing ovarian tumor. J. Clin. Endocrinol. Metab., 1972; 34: 521-530.
11. Goldstein, D., Zuckerman, H., Harpaz, S., et al., Correlation between estradiol and progesterone in cycles with luteal phase deficiency. Fertil. Steril., 1982; 37: 348-354.
12. Kirschner, M.A., The role of hormones in the etiology of human breast cancer. Cancer, 1977; 39: 2716-2726.
13. Odell, W.D. and Swerdloff, R.S., Abnormalities of gonadal function in men. Clin. Endocr., 1978; 8: 149-180.
14. MacDonald, P.C., Madden, J.D., Brenner, P.F., Wilson, J.D. and Siiteri, P.K., Origin of estrogen in normal men and in women with testicular feminization, J. Clin. Endocrinol. Metab., 1979; 49: 905-916.
15. 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 fertilization using the natural menstrual cycle or follicular stimulation with clomiphene citrate and human menopausal gonadotropin, J. In Vitro Fertil. Embryo Transfer, 1985; 2: 123-131.
16. Ratcliffe, W.A., Carter, G.D., Dowsett, M., et al., Oestradiol assays: applications and guidelines for the provision of a clinical biochemistry service, Ann. Clin. Biochem., 1988; 25:466-483.
17. Tietz, N.W. ed., Clinical Guide to Laboratory Tests, 3rd Edition, W.B. Saunders, Co., Philadelphia, 1995: 216-217.
18. USA Center for Disease Control/National Institute of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories", 1984.
19. ICN Guide to Endocrine Testing. Diagnostic Division, ICN Biomedicals, Inc. pp. 2:15-19.

2009-06-05 (Mfg: 052806)



## Estradiol ELISA

Catalog No. ES071S (96 Tests)

### NAME AND INTENDED USE







The Calbiotech, Inc (CBI) Estradiol (E2) ELISA Kit is intended for the quantitative determination of Estradiol (E2) concentration in human serum or plasma.

### SUMMARY AND EXPLANATION OF THE TEST

Estradiol E2 is the most potent natural Estrogen, produced mainly by the ovary, placenta, and in smaller amounts by the adrenal cortex, and the male testes. Estradiol is secreted into the blood stream where 98% bound to sex hormone binding globulin (SHBG). Estrogenic activity is effected via estradiol-receptor complexes which trigger the appropriate response at 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. During pregnancy, maternal serum Estradiol levels increase considerably, to well above the pre-ovulatory peak levels and high levels are sustained throughout pregnancy. Serum Estradiol measurements are a valuable index in evaluating a variety of menstrual dysfunctions such as precocious or delayed puberty in girls and primary and secondary amenorrhea and menopause. Estradiol levels have been reported to be increased in patients with feminizing syndromes, gynaecomastia and testicular tumors. In cases of infertility, serum Estradiol measurements are useful for monitoring induction of ovulation following treatment.

### PRINCIPLE OF THE TEST

The E2 EIA is based on the principle of competitive binding between E2 in the test specimen and E2-HRP conjugate for a constant amount of rabbit anti-Estradiol. In the incubation, goat anti-rabbit IgG-coated wells are incubated with E2 standards, controls, patient samples, Estradiol-HRP Conjugate Reagent and rabbit anti-Estradiol reagent at room temperature for 90 minutes. During the incubation, a fixed amount of HRP-labeled E2 competes with the endogenous E2 in the standard, sample, or quality control serum for a fixed number of binding sites of the specific E2 antibody. E2 peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of E2 in the specimen increases. Unbound E2 peroxidase conjugate is then removed and the wells washed. Next, a solution of TMB Reagent is added and incubated at room temperature for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of stop solution, and the absorbance is measured spectrophotometrically at 450 nm. A standard curve is obtained by plotting the concentration of the standard versus the absorbance.

Cat#: ES071S (96 Tests)  
 For Order and Inquiries, please contact  
 Calbiotech Inc.,  
 10461 Austin Dr, Spring Valley, CA, 91978  
 Tel (619) 660-6162, Fax (619) 660-6970,  
[www.calbiotech.com](http://www.calbiotech.com)  
    
  CEpartner4U, 3951DB; 13. NL.  
 tel: +31 (0)6.516.536.26

MATERIALS PROVIDED	96 Tests
1. Microwells coated with Goat Anti-Rabbit IgG	12x8x1
2. Estradiol Reference Standards: 6 vials (ready to use)	0.5 ml
3. Rabbit Anti-Estradiol Reagent (pink color)	7 ml
4. Estradiol-HRP Conjugate Reagent (blue color)	12 ml
5. Estradiol Control 1, Liquid. (ready to use)	0.5 ml
6. Estradiol Control 2, Liquid. (ready to use)	0.5 ml
7. TMB Reagent	11 ml
8. Stop Solution	11 ml
9. Wash Concentrate 20x: 1 Bottle	25 ml

**MATERIALS NOT PROVIDED**

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

**STORAGE AND STABILITY**

1. Store the kit at 2-8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light.

**WARNINGS AND PRECAUTIONS**

1. Potential biohazardous materials:  
The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
2. This kit is USA FDA exempt product.
3. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. Control sera and sample diluent contain preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

**SPECIMEN COLLECTION AND PREPARATION**

1. Only Human Serum should be used.
2. No special pretreatment of sample is necessary.
3. Serum samples may be stored at 2-8°C for up to 24 hours, and should be frozen at -10°C or lower for longer periods. Do not use grossly hemolyzed or grossly lipemic specimens.
4. Samples containing sodium azide should not be used in the assay.

**REAGENT PREPARATION**

1. All reagents should be brought to room temperature (18-26°C) before use.
2. Samples with expected Estradiol concentrations over 1000 ng/ml may be quantitated by dilution with diluent available from vendor.
3. Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26 °C).

**ASSAY PROCEDURE**

1. Secure the desired number of coated wells in the holder.
2. Dispense 25 µl of standards, specimens and controls into appropriate wells.
3. Dispense 100 µl of Estradiol-HRP Conjugate Reagent into each well.
4. Dispense 50 µl of rabbit anti-Estradiol(E2) reagent to each well.
5. **Thoroughly mix for 30 seconds. It is very important to mix them completely.**
6. Incubate at room temperature (18-25°C) for 90 minutes.
7. Remove liquid from all wells. Wash wells three times with 300 µL of 1X wash buffer. Blot on absorbance paper or paper towel.
8. Dispense 100 µl of TMB Reagent into each well. Gently mix for 10 seconds.
9. Incubate at room temperature (18-25°C) for 20 minutes.
10. Stop the reaction by adding 100 µl of Stop Solution to each well.
11. Gently mix 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
12. Read absorbance at 450 nm with a microtiter well reader within 15 minutes.

**CALCULATION OF RESULTS**

1. Calculate the mean absorbance value ( $A_{450}$ ) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in pg/ml on a linear-linear graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
3. Use the mean absorbance values for each specimen to determine the corresponding concentration of Estradiol in pg/ml from the standard curve.
4. Any values obtained for diluted samples must be further converted by applying the appropriate dilution factor in the calculations.

**EXPECTED VALUES**

Each laboratory should establish its own normal range based on the patient population. The following values could be used as guide line: Males: 10-50 pg/ml, Females: postmenopausal phase 0-30 pg/ml, ovulating 30-400 pg/ml, early follicular 30-100 pg/ml, late follicular 100-400 pg/ml, luteal phase 50-200 pg/ml, pregnant, normal up to 35,000 pg/ml, prepubertal children, normal < 10 pg/ml.

**Example of A Standard Curve**

Results of a typical standard run with optical density readings at 450 nm shown in the Y-axis against Estradiol concentrations shown in the X axis. Note: This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each laboratory must provide its own data and standard curve in each experiment

Estradiol (pg/ml)	Absorbance (450 nm)
0	2.943
10	2.551
30	2.055
100	1.624
300	0.925
1000	0.571

**QUALITY CONTROL**

Good laboratory practice requires that controls are 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. We recommend using Bio-Rad Lyphochek Immunoassay Control Sera as controls. The Estradiol EIA kit also provides with internal controls, Level 1 and 2.

**PERFORMANCE CHARACTERISTICS**

1. **Sensitivity**  
The minimum detectable concentration of the Estradiol ELISA assay as measured by 2 SD from the mean of a zero standard is estimated to be 10 pg/ml.
2. **Precision**  
**Intra-Assay Precision**  
Within-run precision was determined by replicate determinations of four different serum samples in one assay. Within-assay variability is shown below:

Samples	1	2	3	4
# Replicates.	24	24	24	24
Mean Estradiol (pg/ml)	13	73	247	633
S.D.	3	7	10	31
C.V. (%)	24.1	10.3	4.1	4.9

**Inter-Assay Precision**

Between-run precision was determined by replicate measurements of six different serum samples over a series of individually calibrated assays. Between-assay variability is shown below:

Samples	1	2	3	4
# Replicates	20	20	20	20
Mean Estradiol (pg/ml)	14	82	264	691
S.D.	4	8	17	45
C.V. (%)	26.7	10.3	6.4	6.6