REFRENCES

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-17β 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., 197 2; 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. Metabl., 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.

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CALBIOTECH

Estradiol ELISA

Catalog No. ES380S (96 Tests)

NAME AND INTENDED USE

The Calbiotech, Inc Estradiol (E2) ELISA Kit is intended for the quantitative determination of Estradiol (E2) concentration in human serum and plasma. **For Research Use Only. For professional use only. Not for use in diagnostic procedures.**

SUMMARY AND EXPLANATION

Estradiol (E2) is the most potent natural estrogen, critical for reproductive tissue development, bone health, and cardiovascular function. This ELISA detects estradiol in serum or plasma for studies on ovarian cycle regulation, estrogen receptor biology, metabolic health, and neuroendocrine function.

PRINCIPLE OF THE TEST

The E2 ELISA kit is based on the principle of **Delayed competitive binding** assay between E2 in the test specimen and E2 enzyme conjugate for a constant amount of anti-Estradiol monoclonal antibody epitops (Biotin reagent). In the incubation, anti-E2 antibody biotin reagent, E2 standards, controls, and samples are incubated for 45 minutes at room temperature (RT), then E2 enzyme conjugate is added on the top of the reaction mixture and incubation continues for 45 minutes more. 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 of anti-Estradiol Biotin Reagent and E2 peroxidase conjugate is then removed and the wells are 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

MATERIALS PROVIDED		96 TESTS
1.	Microwells coated with Streptavidin	12x8x1
2.	Estradiol Standards set: 6 vials (Ready to use)	0.5 mL
3.	Estradiol Control set: 2 vials (Ready to use)	0.5 mL
4.	Estradiol Biotin Conjugate: 1bottle (ready to use)	7 mL
5.	Estradiol Enzyme Conjugate Concentrate, 20X: 1Vial	0.7 mL
6.	Assay Diluent: 1 bottle (Ready to use)	12 mL
7.	TMB Reagent: 1bottle (Ready to use)	12 mL
8.	Stop Solution: 1 bottle (Ready to use)	12 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 plate 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

Potential biohazardous materials:

1. The standards 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. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. It is recommended that standards, control and serum samples be run in duplicate
5. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION AND PREPARATION

This assay is designed for use with human serum or plasma samples obtained in compliance with applicable laws, regulations, and institutional policies. Handle and store samples using procedures appropriate for research use. Samples may be stored refrigerated (2–8 °C) for up to seven days, or frozen (–20 °C or below) for up to six months. Avoid repetitive freeze–thaw cycles.

REAGENT PREPARATION

1. **20X Enzyme conjugate**: Prepare 1X working solution at 1:20 with assay diluent (e.g. Add 0.1ml of the E2 enzyme conjugate concentrate to 1.9ml of assay diluent)
2. **20X Wash buffer:** Prepare 1X Wash buffer by adding the contents of the bottle (25ml, 20X) to 475ml of distilled or deionized water. Store at room temperature (20-25°C).

ASSAY PROCEDURE

1. Bring all reagents to RT (20-25 °C) before use.
2. Secure the desired number of coated wells in the holder.
3. Dispense 25µl of standards, specimens and controls into appropriate wells.
4. Dispense 50µl of working solution of Estradiol Biotin Reagent into each well.
5. Mix well by placing on shaker for 10 – 20 seconds
6. Incubate at (20-25°C) for 45 minutes.
7. Dispense 100µl of Estradiol Enzyme Reagent to all wells. (Note: Add directly on the top of the Biotin)
8. Mix well by placing on shaker for 10 – 20 seconds.
9. Incubate at (20-25°C) for 45 minutes.
10. Remove liquid from all wells. Wash wells three times with 300 µL of 1X wash buffer. Blot on absorbance paper or paper towel.
11. Dispense 100µl of TMB Reagent into each well. Incubate at (20-25°C) for 20 minutes.
12. Stop the reaction by adding 50 µl of Stop Solution to each well.
13. Gently mix 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
14. Read absorbance at 450 nm with a microplate 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.

Example of A Standard Curve

Estradiol (pg/ml)	Absorbance (450 nm)
0	2.1142
10	1.8638
30	1.5074
100	1.0233
300	0.5265
1000	0.106

LIMITATION OF THE TEST

1. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.