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## Estradiol ELISA

Catalog No. ES180S (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 and 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 Calbiotech, Inc E2 ELISA kit is based on the principle of competitive binding between E2 in the test specimen and E2 enzyme conjugate for a constant amount of anti-Estradiol polyclonal antibody. In the incubation, anti-E2 antibody coated wells are incubated with E2 standards, controls, samples, and E2 enzyme conjugate at room temperature for 60 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 are washed. Next, a solution of TMB Reagent is added and incubated at room temperature for 30 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 polyclonal anti-Estradiol Antibody | 12x8x1   |
| 2. Estradiol Standards: 6 vials (Ready to use)               | 0.5 ml   |
| 3. Estradiol Enzyme conjugate Concentrate, 20X, 1Vial        | 0.7 ml   |
| 4. Assay Diluent , 1 bottle (Ready to use)                   | 12ml     |
| 5. TMB Reagent, 1bottle (Ready to use)                       | 12 ml    |
| 6. 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

Cat#: ES180S (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

**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 Standard set contains 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.

**SPECIMEN COLLECTION AND PREPARATION**

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2-8° C) for 5 days. For long term storage frozen at (-20° C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

**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. **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. Bring all reagents to room temperature (18 – 26 °C) before use..
2. Secure the desired number of coated wells in the holder.
3. Dispense 25 µl of standards, specimens and controls into appropriate wells.
4. Dispense 100 µl of working solution of Estradiol enzyme conjugate into each well.
5. Mix well by placing on shaker for 10 – 20 seconds
6. Incubate at room temperature (18-25°C) for 60 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 30 minutes.
10. Stop the reaction by adding 50 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 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.

**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**

| Estradiol (pg/ml) | Absorbance (450 nm) |
|-------------------|---------------------|
| 0                 | 2.069               |
| 10                | 1.623               |
| 30                | 1.292               |
| 100               | 0.794               |
| 300               | 0.388               |
| 1000              | 0.162               |

**LIMITATION OF THE TEST**

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient history, physical findings and other diagnostic procedures.
2. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

**PERFORMANCE CHARACTERISTICS****1. Sensitivity**

The sensitivity of the assay is 3.94 pg/ml. The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 20 times in the same run.

**2. Correlation with a Reference ELISA kit:**

A total of 60 samples were tested by this kit and a commercially available Estradiol ELISA kit. The linear regression curve was calculated as:  $Y = 0.931 - 2.40$ ,  $r = 0.979$

**3. Precision****Intra-Assay**

| Serum | No. of Replicates | Mean ng/ml | Standard Deviation | Coefficient of Variation (%) |
|-------|-------------------|------------|--------------------|------------------------------|
| 1     | 20                | 29.5       | 2.54               | 8.6                          |
| 2     | 20                | 143.6      | 12.75              | 8.9                          |
| 3     | 20                | 198.4      | 13.95              | 7.0                          |

**Inter-Assay**

| Serum | No. of Replicates per test | Mean pg/ml | Standard Deviation | Coefficient of Variation (%) |
|-------|----------------------------|------------|--------------------|------------------------------|
| 1     | 12                         | 29.4       | 2.538              | 8.6                          |
| 2     | 12                         | 147.9      | 7.042              | 4.8                          |
| 3     | 12                         | 202.6      | 8.179              | 4.0                          |

**4. Linearity**

Two different patient samples were diluted with the "0" calibrator to 1:2, 1:4, 1:8. Estradiol values were calculated and results were corrected with the dilution factor.

| Original Value |         | Percentage of Recovery |       |       |
|----------------|---------|------------------------|-------|-------|
| Serum          | (pg/ml) | 1/2                    | 1/4   | 1/8   |
| 1              | 186.7   | 103.3                  | 109.4 | 106.5 |
| 2              | 288.8   | 110.7                  | 93.3  | 109.9 |