**SUMMARY AND EXPLANATION OF THE TEST**

Measurement of estradiol in serum or plasma is considered to be the most reliable way to assess its rate of production.

Estradiol (18)-estradiol is a steroid hormone (molecular weight of 272.3 daltons), which circulates predominantly protein-bound. In addition to estradiol, other natural steroidal estrogens include estrone, estradiol and their metabolites. Natural estrogens are hormones secreted principally by the ovarian follicles and also by the adrenal cortex, uterus, and placenta and, in males, by the testes. Exogenous estradiol (either natural or synthetic) is also administered at varying doses, all of which pharmacologic responses usually produced by endogenous estrogens.

Estrogenic hormone levels vary at varying rates during the menstrual cycle throughout the period of ovarian activity. During pregnancy, the placenta becomes the main source of estrogens. At menopause, ovarian secretion of estrogens declines at varying rates. The gonadotropins of the anterior pituitary gland regulate secretion of the ovarian hormones, estradiol and progesterone; hypothalamic secretion of pituitary gonadotropin production is in turn regulated by plasma concentrations of the estrogens and progesterone. This complex feedback system results in the cyclic phenomenon of ovulation and menstruation. Estradiol determinations have proved of value in a variety of contexts, including the investigation of precocious puberty in girls and gynecomastia in men. Its principal uses have been in the differential diagnosis of amenorrhea and in the monitoring of ovulation induction.

This kit uses a specific anti-estradiol antibody, and does not require prior sample extraction of serum or plasma. Cross-reactivity to other naturally occurring and structurally related steroids is low. The employment of several serum references of known estradiol concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with estradiol concentration.

**PRINCIPLE**

Delayed Competitive Enzyme Immunoassay (Type 9):

The essential reagents required for this enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen. Upon mixing the biotinylated antibody with a serum containing the antigen, a reaction results between the antigen and the antibody. The interaction is illustrated by the following equations:

\[ AgAbBtn = \text{sandwich complex bound to the solid} \]

\[ AgAbBtn + \text{EnzAgAbBtn} = \text{immobilized complex} \]

AccuBind™ Estradiol (E2) ELISA

Product Code: 4925-30

**REQUIRED BUT NOT PROVIDED:**

- 50 µl and 500 µl with a precision
- 8. Cover and incubate for 90 minutes at room temperature.
- Add 350 µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes.
- Automatic or manual plate washer can be used. Follow the manufacturer’s instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash buffer. Decant the wash and repeat two (2) additional times.

**SPECIMEN COLLECTION AND PREPARATION**

- Dilute the samples suspected of concentrations higher than 3000 pg/ml 1:5 and 1:10 with estradiol 0 pg/ml calibrator or male patient serum pools with a known low value for estradiol.

- The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

**CALCULATION OF RESULTS**

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding estradiol concentration in pg/ml on a graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve. To determine the concentration of estradiol for an unknown specimen, read the absorbance for the unknown specimen on the x-axis. The y-axis value represents the estradiol concentration in pg/ml.

**PRECAUTIONS**

- Do not shake the plate after substrate addition.
- DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION.
- Incubate at room temperature for 20 minutes.
- Add 0.050 ml (50 µl) of stop solution to each well and gently:

**NOTE:** Dilute the samples suspected of concentrations higher than 3000 pg/ml 1:5 and 1:10 with estradiol 0 pg/ml calibrator or male patient serum pools with a known low value for estradiol.

**QUALITY CONTROL**

- All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1 & 2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, “ Biosafety in Microbiological and Biomedical Laboratories,” 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

**SPECIMEN COLLECTION AND PREPARATION**

The specimens shall be blood; serum or heparinized plasma in type and taken with the usual precautions in the collection of venipuncture samples. For accurate comparison to establish normal values, a fasting morning serum sample should be obtained. The blood should be collected in a red top (with or without additional) venipuncture tube(s) or in plastic tube(s) containing heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the samples may be stored at temperatures of 0°-10°C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml of the specimen is required.

**REAGENT PREPARATION**

1. Wash Buffer:

   Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at room temperature (20-27°C) for up to 60 days.

   **Note:** Do not use the working substrate if it looks blue.

2. Wash Procedure:

   **Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 27°C).**

   1. Format the microplates' wells for each serum reference, control and specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag and store at 2-8°C.
   2. Pipette 0.025 ml (25 µL) of the appropriate serum reference, control and specimen into the assigned well.
   3. Add 0.050 ml (50 µl) of the Estradiol Biotin Reagent to all wells.
   4. Swirl the microplate gently for 20-30 seconds to mix.
   5. Cover and incubate for 30 minutes at room temperature.
   6. Add 0.050 ml (50 µl) of Estradiol Enzyme Reagent to all wells.
   7. Swirl the microplate gently for 20-30 seconds to mix.

   **Note:** Dilute the samples suspected of concentrations higher than 3000 pg/ml 1:5 and 1:10 with estradiol 0 pg/ml calibrator or male patient serum pools with a known low value for estradiol.

   **CALCULATION OF RESULTS**

A dose response curve is used to ascertain the concentration of estradiol in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding estradiol concentration in pg/ml on a graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve.

To determine the concentration of estradiol for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in pg/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.20) intersects the dose response curve at (160pg/ml) estradiol concentration (See Figure 1).

### EXAMPLE 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number</th>
<th>Well</th>
<th>Axis (A)</th>
<th>Axis (B)</th>
<th>Mean (pg/ml)</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cal A</td>
<td></td>
<td>B1</td>
<td>2.268</td>
<td></td>
<td>2.256</td>
<td>0</td>
</tr>
<tr>
<td>Cal B</td>
<td></td>
<td>C1</td>
<td>1.839</td>
<td></td>
<td>1.849</td>
<td>20</td>
</tr>
<tr>
<td>Cal C</td>
<td></td>
<td>E1</td>
<td>1.409</td>
<td></td>
<td>1.426</td>
<td>100</td>
</tr>
<tr>
<td>Cal D</td>
<td></td>
<td>G1</td>
<td>1.017</td>
<td></td>
<td>1.003</td>
<td>250</td>
</tr>
<tr>
<td>Cal E</td>
<td></td>
<td>A2</td>
<td>0.698</td>
<td></td>
<td>0.723</td>
<td>500</td>
</tr>
<tr>
<td>Cal F</td>
<td></td>
<td>C2</td>
<td>0.480</td>
<td></td>
<td>0.487</td>
<td>1500</td>
</tr>
<tr>
<td>Cal G</td>
<td></td>
<td>E2</td>
<td>0.390</td>
<td></td>
<td>0.388</td>
<td>3000</td>
</tr>
<tr>
<td>Patl1</td>
<td></td>
<td>G2</td>
<td>1.202</td>
<td></td>
<td>1.202</td>
<td>160</td>
</tr>
</tbody>
</table>

### Table 1

**Expected Values for the Estradiol Test System**

<table>
<thead>
<tr>
<th>Estradiol Value /1000 pg/ml</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>160</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2

**Within Assay Precision (Values in pg/ml)**

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>C</th>
<th>E</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>20</td>
<td>85.9</td>
<td>7.6</td>
<td>8.8%</td>
</tr>
<tr>
<td>Normal</td>
<td>20</td>
<td>260.5</td>
<td>20.3</td>
<td>7.8%</td>
</tr>
<tr>
<td>High</td>
<td>20</td>
<td>465.3</td>
<td>37.7</td>
<td>6.8%</td>
</tr>
</tbody>
</table>

### Table 3

**Between Assay Precision (Values in pg/ml)**

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>C</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>10</td>
<td>89.3</td>
<td>8.2</td>
<td>9.2%</td>
</tr>
<tr>
<td>Normal</td>
<td>10</td>
<td>245.5</td>
<td>23.7</td>
<td>9.7%</td>
</tr>
<tr>
<td>High</td>
<td>10</td>
<td>467.2</td>
<td>38.3</td>
<td>8.2%</td>
</tr>
</tbody>
</table>

As measured in ten experiments in duplicate over a ten day period.

### Table 4

**Least Square Regression Analysis**

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean (x)</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>This Method (Y)</td>
<td>139</td>
<td>y = 10.985(x)^0.979</td>
</tr>
</tbody>
</table>

### Table 5

**Expected Ranges of Values**

In agreement with established reference intervals for a “normal” adult population and females during gestation the expected ranges for the Estradiol AccuBind™ ELISA Test System are detailed in Table 1.

**Q.C. PARAMETERS**

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 0 pg/ml should be ≤ 1.3.
2. Four out of six quality control pools should be within the established ranges.

### Risk Analysis

**A. Assay Performance**

1. It is important that the time of reaction in each well is held constant for reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
4. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution.

### References