Estradiol ELISA

INTENDED USE
For the direct quantitative determination of estradiol by an enzyme immunoassay in human serum.

PRINCIPLE OF THE TEST
The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabelled estradiol (present in standards, controls and patient samples) and an enzyme-labelled estradiol (conjugate) for a limited number of antibody binding sites on the microplate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is monitored with absorption of the stopping solution. The absorbance is measured on a microplate reader. The intensity of the colour formed is inversely proportional to the concentration of the unlabelled estradiol in the sample. A set of standards is used to plot a standard curve from which the amount of estradiol in patient samples and controls can be directly read.

CLINICAL APPLICATIONS
Estradiol is one of the main components of naturally occurring estrogens and is the major estrogen secreted during the menstrual cycle. The serum levels of estradiol are low during the follicular phase rising gradually until about one day before ovulation when a marked rise in the estradiol level occurs (Ovulatory Peak). The estradiol level falls rapidly at, or right of the stopping solution. The absorbance is measured on a microplate reader. The intensity of the colour formed is inversely proportional to the concentration of the unlabelled estradiol in the sample. A set of standards is used to plot a standard curve from which the amount of estradiol in patient samples and controls can be directly read.

PROCEDURAL CAUTIONS AND WARNINGS
1. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
2. It’s recommended to all customers to prepare their own control materials or serum pools that should be included in every run at a high and low level for assessing the performance of the kit.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only calibrator A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
5. The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of heterophile antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient’s background including the frequency of exposure to animals/ products if false results are suspected.
6. This assay should not be used for patients being treated with the drug fulvestrant (Faslodex®) which cross reacts with estradiol and could lead to a falsely elevated test result.

SAFETY PRECAUTIONS AND STORAGE

Approximately 0.2 mL of serum is required per duplicate determination. Gently mix, if necessary, of an appropriately labelled tube and allow to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN COLLECTION AND STORAGE

1. Estradiol-Biotin: Avidin-Horseradish Peroxidase (HRP) Conjugate Concentrate — Requires Preparation
2. Calibrator E 800 pg/mL 0.5 mL
3. Calibrator D 300 pg/mL 0.5 mL
4. Calibrator C 100 pg/mL 0.5 mL
5. Calibrator B 20 pg/mL 0.5 mL
6. Calibrator A 0 pg/mL 2.0 mL
7. TMB Substrate — Ready To Use
8. Buffer A — Ready To Use
9. Buffer B — Ready To Use
10. Wash Buffer Concentrate — Ready To Use

Approximately 0.2 mL of serum is required per duplicate determination. Gently mix, if necessary, of an appropriately labelled tube and allow to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

This assay is a direct system; no specimen pretreatment is necessary.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 50, 100, 150 and 300 μL
2. Disposable pipette tips
3. Diluent deionized water
4. Plate shaker
5. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater (see assay procedure step 10)

REAGENTS PROVIDED

1. Rabbit Anti-Estradiol Antibody-Coated Break-Apart Well Microplate — Ready To Use
2. Controls — Ready To Use
3. Distilled or deionized water
4. Disposable pipette tips
5. Wash Buffer Concentrate — Ready To Use
6. Assay Buffer — Ready To Use
7. TMB Substrate — Ready To Use
8. Stopping Solution — Ready To Use

* Listed below are approximate concentrations, please refer to vial labels for the acceptable ranges.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Volume/Vial</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 mg/mL</td>
<td>2.0 mL</td>
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<tr>
<td>0.5 mg/mL</td>
<td>0.5 mL</td>
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<tr>
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<td>0.5 mL</td>
</tr>
<tr>
<td>0.5 mg/mL</td>
<td>0.5 mL</td>
</tr>
</tbody>
</table>

Storage: Refrigerate at 2–8°C
Stability: 12 months in unopened vials or as indicated on vial. Once opened, the controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

Controls — Ready To Use
Contents: Two vials containing estradiol in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of estradiol. Refer to vial labels for the acceptable range.

Volume: 0.5 mL/vial
Storage: Refrigerate at 2–8°C
Stability: 12 months in unopened vials or as indicated on vial. Once opened, the controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

Wash Buffer Concentrate — Requires Preparation
Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.

Volume: 6 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.

Preparation: Dilute 1:10 in distilled or deionized water and store frozen. If the whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.

Contents: One bottle containing tetramethyl benzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.

Volume: 16 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.

Preparation:

1. Estradiol Calibrators — Ready To Use
Contents: Six vials containing estradiol in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of estradiol.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Volume/Vial</th>
</tr>
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<tbody>
<tr>
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</tr>
<tr>
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<td>0.5 mL</td>
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<tr>
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<td>0.5 mL</td>
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<td>0.0 mg/mL</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>0.0 mg/mL</td>
<td>0.5 mL</td>
</tr>
</tbody>
</table>

Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.

Preparation:

1. Stopping Solution — Ready To Use
Contents: One bottle containing 1M sulfuric acid.

Volume: 6 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.
**CALCULATIONS**

1. Calculate the mean optical density of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean concentrations on the X-axis.
3. Pipette 50 μL of each calibrator, control, and specimen sample into correspondingly labelled wells in duplicate.
4. Pipette 100 μL of the conjugate working solution into each well. (We recommend using a multichannel pipette.)
5. Incubate on a plate shaker (approximately 200 rpm) within 20 minutes after addition of the stopping solution.
6. Wash the wells 3 times with 300 μL of diluted wash buffer.
7. Pipette 150 μL of TMB substrate into each well at timed intervals.
8. Incubate on a plate shaker (approximately 200 rpm) for 1 hour at room temperature.
9. Pipette 100 μL of the conjugate working solution into each well at the same timed intervals as in step 7.
10. Read the plate on a microplate reader at 450 nm filter is unavailable, a 405 or 415 nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.

**SPECIFICITY (CROSS-REACTIVITY)**

The following compounds were tested for cross-reactivity with the Direct Estradiol ELISA kit with estradiol cross-reacting at 100%.

- If the OD exceeds the upper limit of detection or if a 450 nm filter is unavailable, a 405 or 415 nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.
- The detection limit is defined as the concentration of estradiol needed to give a B/B0 values equivalent to the point where B = 0.902, 0.883, 0.893, 300 μL of diluted wash buffer per well and tip the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended.)
- Intra- and Inter-Assay Precision.

**LINEARITY**

Three human serum samples were diluted with calibrator A. The linearity results (in pg/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>OD 1</th>
<th>OD 2</th>
<th>Mean OD</th>
<th>Value (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.001</td>
<td>1.952</td>
<td>1.976</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>1.716</td>
<td>1.775</td>
<td>1.746</td>
<td>20</td>
</tr>
<tr>
<td>C</td>
<td>1.397</td>
<td>1.356</td>
<td>1.377</td>
<td>100</td>
</tr>
<tr>
<td>D</td>
<td>0.952</td>
<td>0.883</td>
<td>0.893</td>
<td>300</td>
</tr>
<tr>
<td>E</td>
<td>0.812</td>
<td>0.702</td>
<td>0.657</td>
<td>800</td>
</tr>
<tr>
<td>F</td>
<td>0.365</td>
<td>0.368</td>
<td>0.367</td>
<td>3200</td>
</tr>
</tbody>
</table>

**TYPICAL TABULATED DATA**

Sample data only. Do not use to calculate results.

**TYPICAL CALIBRATOR CURVE**

Sample curve only. Do not use to calculate results.

**ASSAY PROCEDURE**

**Performance Characteristics**

**Sensitivity**

The detection limit is defined as the concentration of estradiol needed to give a B/B0 values equivalent to the point where B = 0.902, 0.883, 0.893. Based on 20 replicate analyses of standard A, the sensitivity is 10 pg/mL.

**Specificity (Cross-Reactivity)**

The following compounds were tested for cross-reactivity with the Direct Estradiol ELISA kit with estradiol cross-reacting at 100%.

Steroid % Cross Reactivity

<table>
<thead>
<tr>
<th>Steroid</th>
<th>% Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>100</td>
</tr>
<tr>
<td>Estrone</td>
<td>1.6</td>
</tr>
<tr>
<td>Estriol</td>
<td>1.3</td>
</tr>
<tr>
<td>Progesterone</td>
<td>0.1</td>
</tr>
<tr>
<td>Cortisol</td>
<td>0.1</td>
</tr>
</tbody>
</table>

This assay should not be used for patients being treated with the drug fulvestrant (Faslodex®) which cross reagents with estradiol and could lead to a falsely elevated test result.

**Intra-Assay Precision**

Three samples were assayed ten times each on the same calibrator curve. The results (in pg/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>85.624</td>
<td>7.846</td>
<td>9.3</td>
</tr>
<tr>
<td>2</td>
<td>355.735</td>
<td>32.372</td>
<td>9.1</td>
</tr>
<tr>
<td>3</td>
<td>1104.385</td>
<td>51.243</td>
<td>4.6</td>
</tr>
</tbody>
</table>

**INTER-ASSAY PRECISION**

Three samples were assayed ten times. The results (in pg/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>82.044</td>
<td>8.286</td>
<td>10.1</td>
</tr>
<tr>
<td>2</td>
<td>324.623</td>
<td>31.838</td>
<td>9.8</td>
</tr>
<tr>
<td>3</td>
<td>1153.301</td>
<td>71.505</td>
<td>6.2</td>
</tr>
</tbody>
</table>

**Recovery**

Three human serum samples were spiked with defined amounts of estradiol. The recovery results (in pg/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Obs. Result</th>
<th>Exp. Result</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>43.312</td>
<td>49.147</td>
<td>116.2</td>
</tr>
<tr>
<td>B</td>
<td>324.067</td>
<td>330.284</td>
<td>109.2</td>
</tr>
<tr>
<td>C</td>
<td>638.328</td>
<td>659.427</td>
<td>112.1</td>
</tr>
<tr>
<td>D</td>
<td>125.661</td>
<td>128.051</td>
<td>106.6</td>
</tr>
<tr>
<td>E</td>
<td>415.680</td>
<td>414.146</td>
<td>102.6</td>
</tr>
<tr>
<td>F</td>
<td>576.160</td>
<td>583.051</td>
<td>96.3</td>
</tr>
<tr>
<td>G</td>
<td>336.297</td>
<td>413.581</td>
<td>114.8</td>
</tr>
<tr>
<td>H</td>
<td>747.791</td>
<td>760.214</td>
<td>100.6</td>
</tr>
<tr>
<td>I</td>
<td>765.287</td>
<td>783.513</td>
<td>93.2</td>
</tr>
</tbody>
</table>

**Expected Values**

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values. The results of an expected range study with apparently normal healthy subjects yielded the following results (all values are reported in pg/mL):

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Mean</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>10.0</td>
<td>2.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Progesterone</td>
<td>0.1</td>
<td>0.01</td>
<td>10.0</td>
</tr>
</tbody>
</table>

**REFERENCES**


**Symbols**

- **Calibrator OD 1 OD 2 Mean OD Value (pg/mL)**
- **TYPICAL CALIBRATOR CURVE**
- **Sample OD (450 nm) Estradiol (pg/mL)**
- **OD 450 nm**
- **Calibrator curve.**
- **OD (450 nm)**
- **Estradiol (pg/mL)**
- **Linearity results (in pg/mL) are tabulated below:**
- **Expected Values**
- **In conclusion**
- **References**
- **Sample curve only. Do not use to calculate results.**
- **Sample data only. Do not use to calculate results.**