17β-ESTRADIOL (SERUM/PLASMA) ASSAY LAYOUT SHEET
for use with Enzo Life Sciences Catalog Nos. ADI-900-174 & ADI-901-174

• DILUTION TABLE FOR MAKING STANDARDS 1-7:

<table>
<thead>
<tr>
<th>Std.</th>
<th>Assay Buffer Vol. (µL)</th>
<th>Vol. Added (µL)</th>
<th>17β-Estradiol Conc. (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Int.</td>
<td>990</td>
<td>10, Stock</td>
<td>3000</td>
</tr>
<tr>
<td>1</td>
<td>800</td>
<td>400, Int.</td>
<td>1000</td>
</tr>
<tr>
<td>2</td>
<td>500</td>
<td>500, Std. 1</td>
<td>500</td>
</tr>
<tr>
<td>3</td>
<td>500</td>
<td>500, Std. 2</td>
<td>250</td>
</tr>
<tr>
<td>4</td>
<td>500</td>
<td>500, Std. 3</td>
<td>125</td>
</tr>
<tr>
<td>5</td>
<td>500</td>
<td>500, Std 4</td>
<td>62.5</td>
</tr>
<tr>
<td>6</td>
<td>500</td>
<td>500, Std 5</td>
<td>31.3</td>
</tr>
<tr>
<td>7</td>
<td>500</td>
<td>500, Std 6</td>
<td>15.6</td>
</tr>
</tbody>
</table>

• ASSAY PROTOCOL FLOW CHART:

<table>
<thead>
<tr>
<th>Well I.D.:</th>
<th>Blank A1, B1</th>
<th>TA C1, D1</th>
<th>NSB E1, F1</th>
<th>Bo G1, H1</th>
<th>Stds. A2-F3</th>
<th>Samples G3-H12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Buffer</td>
<td>---</td>
<td>---</td>
<td>150 µL</td>
<td>100 µL</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Std. and/or Sample</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>100 µL</td>
<td>100 µL</td>
<td>---</td>
</tr>
<tr>
<td>Conjugate</td>
<td>---</td>
<td>---</td>
<td>50 µL</td>
<td>50 µL</td>
<td>50 µL</td>
<td>50 µL</td>
</tr>
<tr>
<td>Antibody</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>50 µL</td>
<td>50 µL</td>
<td>50 µL</td>
</tr>
<tr>
<td>Incub. 2 hrs. @ RT, shaking (sealed)</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Asp. &amp; Wash 3 x 400 µL</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Conjugate</td>
<td>---</td>
<td>5 µL*</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Substrate</td>
<td>200 µL</td>
<td>200 µL</td>
<td>200 µL</td>
<td>200 µL</td>
<td>200 µL</td>
<td>200 µL</td>
</tr>
<tr>
<td>Incub. 1 hour @ RT</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>50 µL</td>
<td>50 µL</td>
<td>50 µL</td>
<td>50 µL</td>
<td>50 µL</td>
<td>50 µL</td>
</tr>
<tr>
<td>read @ 405 nm</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>
### 17β-Estradiol Plate Layout:

<table>
<thead>
<tr>
<th></th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
<th>A5</th>
<th>A6</th>
<th>A7</th>
<th>A8</th>
<th>A9</th>
<th>A10</th>
<th>A11</th>
<th>A12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Blank</td>
<td>A2</td>
<td>Std 1</td>
<td>A3</td>
<td>Std 5</td>
<td>A4</td>
<td>A5</td>
<td>A6</td>
<td>A7</td>
<td>A8</td>
<td>A9</td>
<td>A10</td>
</tr>
<tr>
<td>B1</td>
<td>Blank</td>
<td>B2</td>
<td>Std 1</td>
<td>B3</td>
<td>Std 5</td>
<td>B4</td>
<td>B5</td>
<td>B6</td>
<td>B7</td>
<td>B8</td>
<td>B9</td>
<td>B10</td>
</tr>
<tr>
<td>C1</td>
<td>TA</td>
<td>C2</td>
<td>Std 2</td>
<td>C3</td>
<td>Std 6</td>
<td>C4</td>
<td>C5</td>
<td>C6</td>
<td>C7</td>
<td>C8</td>
<td>C9</td>
<td>C10</td>
</tr>
<tr>
<td>D1</td>
<td>TA</td>
<td>D2</td>
<td>Std 2</td>
<td>D3</td>
<td>Std 6</td>
<td>D4</td>
<td>D5</td>
<td>D6</td>
<td>D7</td>
<td>D8</td>
<td>D9</td>
<td>D10</td>
</tr>
<tr>
<td>E1</td>
<td>NSB</td>
<td>E2</td>
<td>Std 3</td>
<td>E3</td>
<td>Std 7</td>
<td>E4</td>
<td>E5</td>
<td>E6</td>
<td>E7</td>
<td>E8</td>
<td>E9</td>
<td>E10</td>
</tr>
<tr>
<td>F1</td>
<td>NSB</td>
<td>F2</td>
<td>Std 3</td>
<td>F3</td>
<td>Std 7</td>
<td>F4</td>
<td>F5</td>
<td>F6</td>
<td>F7</td>
<td>F8</td>
<td>F9</td>
<td>F10</td>
</tr>
<tr>
<td>G1</td>
<td>Bo</td>
<td>G2</td>
<td>Std 4</td>
<td>G3</td>
<td>G4</td>
<td>G5</td>
<td>G6</td>
<td>G7</td>
<td>G8</td>
<td>G9</td>
<td>G10</td>
<td>G11</td>
</tr>
<tr>
<td>H1</td>
<td>Bo</td>
<td>H2</td>
<td>Std 4</td>
<td>H3</td>
<td>H4</td>
<td>H5</td>
<td>H6</td>
<td>H7</td>
<td>H8</td>
<td>H9</td>
<td>H10</td>
<td>H11</td>
</tr>
</tbody>
</table>

Kit Lot No. ________________  Exp. Date ________________  Date ________________  Tech. ________________

1st Incub.:  Start Time ______  Temp. ______  Notes: ______________________________________________
             End Time ______  Temp. ______  ______________________________________________

2nd Incub.:  Start Time ______  Temp. ______  ______________________________________________
             End Time ______  Temp. ______  ______________________________________________