REFERENCES

Mouse/Rat Estradiol ELISA

INTENDED USE
For Research Use Only. Not for use in diagnostic procedures.

MATERIALS PROVIDED

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwells coated with polyclonal anti-Estradiol Antibody</td>
<td>12x8x1</td>
</tr>
<tr>
<td>Estradiol Standards: 6 vials (Ready to use)</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>Estradiol Enzyme conjugate Concentrate, 20X, 1Vial</td>
<td>0.7 ml</td>
</tr>
<tr>
<td>Assay Diluent, 1 bottle (Ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>TB Reagent, 1 bottle (Ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>Stop Solution, 1 bottle (Ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>Wash Concentrate 20X, 1 Bottle</td>
<td>25 ml</td>
</tr>
</tbody>
</table>

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
1. For Research Use Only. Not for use in diagnostic procedures.
2. For laboratory use.
3. Potential biohazardous materials:
   The kit contains animal and/or human source components. All the human components 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.
   All the animal products, if any, have been derived from animals of US origin and processed in USDA licensed facilities.
4. 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.
5. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
6. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

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SPECIMEN COLLECTION AND PREPARATION

1. Collect blood specimens and separate the serum or plasma immediately.
2. Typically, specimens may be stored refrigerated at (2-8°C) for 5 days. For long term, samples may be stored frozen at temperature of -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 0.1 ml of the E2 enzyme conjugate concentrate to 1.9 ml 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 (20-25°C).

ASSAY PROCEDURE

1. Bring all reagents to room temperature (20-25°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 reagent of Estradiol enzyme conjugate into each well.
5. Mix well by placing on shaker for 10 – 20 seconds
6. Incubate at room temperature (20-25°C) for 120 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 (20-25°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.

Example of Standard Curve

<table>
<thead>
<tr>
<th>Estradiol (pg/ml)</th>
<th>Absorbance (450 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.06</td>
</tr>
<tr>
<td>3</td>
<td>1.82</td>
</tr>
<tr>
<td>10</td>
<td>1.67</td>
</tr>
<tr>
<td>30</td>
<td>1.31</td>
</tr>
<tr>
<td>100</td>
<td>0.85</td>
</tr>
<tr>
<td>300</td>
<td>0.41</td>
</tr>
</tbody>
</table>

LIMITATION OF THE TEST

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

Kit Name: Mouse/Rat Estradiol
Lot # ESG6183

<table>
<thead>
<tr>
<th>Component</th>
<th>Part Number</th>
<th>Lot Number</th>
<th>Expiration Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol Double-Layer Coated Plate</td>
<td>ES296AB</td>
<td>AB2716</td>
<td>2023-04-28</td>
</tr>
<tr>
<td>Estradiol Standard 1</td>
<td>ES788SS-1</td>
<td>SS2731</td>
<td>2022-11-30</td>
</tr>
<tr>
<td>Estradiol Standard 2</td>
<td>ES788SS-2</td>
<td>SS2731</td>
<td>2022-11-30</td>
</tr>
<tr>
<td>Estradiol Standard 3</td>
<td>ES788SS-3</td>
<td>SS2731</td>
<td>2022-11-30</td>
</tr>
<tr>
<td>Estradiol Standard 4</td>
<td>ES788SS-4</td>
<td>SS2731</td>
<td>2022-11-30</td>
</tr>
<tr>
<td>Estradiol Standard 5</td>
<td>ES788SS-5</td>
<td>SS2731</td>
<td>2022-11-30</td>
</tr>
<tr>
<td>Estradiol Standard 6</td>
<td>ES788SS-6</td>
<td>SS2731</td>
<td>2022-11-30</td>
</tr>
<tr>
<td>Estradiol Enzyme conjugate Concentrate, 20X: 1 vial</td>
<td>ES660EG</td>
<td>EG5295</td>
<td>2022-11-30</td>
</tr>
<tr>
<td>Assay Diluent, 1 bottle (Ready to use)</td>
<td>ES622AD</td>
<td>AD0759</td>
<td>2022-11-30</td>
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<tr>
<td>TMB Substrate: 1 bottle (ready to use)</td>
<td>CC956TM</td>
<td>TM0192</td>
<td>2024-06-05</td>
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<tr>
<td>Stop Solution: 1 bottle (ready to use)</td>
<td>CC604SS</td>
<td>SS0162</td>
<td>2023-11-13</td>
</tr>
<tr>
<td>Wash Concentrate, 20X: 1 bottle</td>
<td>CC601WC</td>
<td>W20134</td>
<td>2023-12-03</td>
</tr>
</tbody>
</table>

This Standard Curve was obtained on the date of performance testing of the above components. It is for the purpose of illustration only. Each laboratory should establish its own standard curve.

Pass ☑️
Reject □

Date: 2021-02-09

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