Mouse/Rat Testosterone ELISA

Catalog Number SE120089
Storage Temperature 2–8 °C

Product Description
Testosterone (17β-hydroxyandrost-4-ene-3-one) is a C19 steroid with an unsaturated bond between C-4 and C-5, a ketone group at C-3 and a hydroxyl group in the β position at C-17. This steroid hormone has a molecular weight of 288.4. Testosterone is the most important androgen secreted into the blood. In males, testosterone is secreted primarily by the Leydig cells of the testes; in females ~50% of circulating testosterone is derived from peripheral conversion of androstenedione, ~25% from the ovary, and ~25% from the adrenal glands. Testosterone is responsible for the development of secondary male sex characteristics and its measurements are helpful in evaluating the hypogonadal states. In women, high levels of testosterone are generally found in hirsutism and virilization, polycystic ovaries, ovarian tumors, adrenal tumors, and adrenal hyperplasia. In male, high levels of testosterone are associated to the hypothalamic pituitary unit diseases, testicular tumors, congenital adrenal hyperplasia, and prostate cancer. Low levels of testosterone can be found in patients with the following diseases: hypopituitarism, Klinefelter’s syndrome, testicular feminization, orchidectomy and cryptorchidism, enzymatic defects, and some autoimmune diseases.

Thus, the amount of Testosterone peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of testosterone in the specimen increases. Unbound Testosterone peroxidase conjugate is then removed and the wells washed. Next, a solution of TMB Reagent is then added and incubated at room temperature for 15 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.

Components

<table>
<thead>
<tr>
<th>Materials Provided</th>
<th>96 Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwell coated with Goat Anti-Rabbit IgG</td>
<td>12 x 8 x 1</td>
</tr>
<tr>
<td>Standard: 6 vials (ready to use)</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Rabbit Anti-Testosterone Reagent (ready to use)</td>
<td>7 mL</td>
</tr>
<tr>
<td>Assay Diluent: 1 bottle (Ready to Use)</td>
<td>12 mL</td>
</tr>
<tr>
<td>Enzyme Conjugate Conc. (20x): 1 Vial</td>
<td>0.7 mL</td>
</tr>
<tr>
<td>TMB Substrate: 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 Buffer (20x): 1 bottle</td>
<td>25 mL</td>
</tr>
</tbody>
</table>

Reagents and Equipment Required but Not Provided.
1. Distilled or deionized water.
2. Precision pipettes.
3. Disposable pipette tips
4. Microplate well reader capable of reading absorbance at 450 nm
5. Absorbent paper or paper towel
6. Graph paper

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.
Preparation Instructions
Sample Preparation
1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2–8 °C) for 5 days. If storage time exceeds 5 days, store 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.
5. Do not use grossly lipemic specimens.

20x Enzyme Conjugate
Prepare 1x working solution by diluting 20-fold with assay diluent as needed (e.g., 0.1 mL of the 20x Enzyme Conjugate in 1.9 mL of Assay Diluent is sufficient for 20 wells). The diluted conjugate has to be used the same day.

20x Wash Buffer Concentrate
Prepare 1x wash buffer by adding the contents of the bottle to 475 mL of distilled water. Store 1x Wash buffer at room temperature.

Storage/Stability
Store the kit at 2–8 °C. Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit. Do not expose reagents to heat, sun, or strong light.

Procedure
Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

It is recommended that serum samples be run in duplicate

Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.
Results

1. Calculate the mean absorbance value (A450) 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 ng/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 testosterone in ng/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.

<table>
<thead>
<tr>
<th>Testosterone (ng/mL)</th>
<th>Absorbance (450 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.38</td>
</tr>
<tr>
<td>0.1</td>
<td>1.75</td>
</tr>
<tr>
<td>0.5</td>
<td>1.02</td>
</tr>
<tr>
<td>2.0</td>
<td>0.59</td>
</tr>
<tr>
<td>6.0</td>
<td>0.34</td>
</tr>
<tr>
<td>18.0</td>
<td>0.17</td>
</tr>
</tbody>
</table>

References

6. USA Center for Disease Control/National Institute of Health Manual, “Biosafety in Microbiological and Biomedical Laboratories” 84