Product Information

Testosterone ELISA

Catalog Number SE120119
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

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, Kliphefelter’s syndrome, testicular feminization, orchidectomy and cryptorchidism, enzymatic defects, and some autoimmune diseases.

The Testosterone ELISA kit is designed for the quantitative determination of total testosterone concentration in human serum or plasma. It is based on the principle of competitive binding between testosterone in the test specimen and testosterone-HRP conjugate for a constant amount of mouse anti-testosterone. In the incubation, mouse anti-testosterone coated wells are incubated with 25 µL of testosterone standards, controls, samples, and 100 µL of testosterone-HRP conjugate reagent at room temperature for 60 minutes. During the incubation, a fixed amount of HRP-labeled testosterone competes with the endogenous testosterone in the standard, sample, or quality control serum for a fixed number of binding sites of the specific testosterone antibody.

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 Mouse Anti-Testosterone</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>Enzyme Conjugate (20x): 1 bottle</td>
<td>0.7 mL</td>
</tr>
<tr>
<td>Assay Diluent: 1 bottle (ready to Use)</td>
<td>12 mL</td>
</tr>
<tr>
<td>TMB Substrate: 1 bottle (ready to use)</td>
<td>12 mL</td>
</tr>
<tr>
<td>Wash Buffer (20x): 1 bottle</td>
<td>25 mL</td>
</tr>
<tr>
<td>Stop Solution: 1 bottle (ready to use)</td>
<td>12 mL</td>
</tr>
</tbody>
</table>

Reagents and Equipment Required but Not Provided.
1. Distilled or deionized water
2. Precision pipettes. Disposable pipette tips
3. ELISA reader capable of reading absorbance at 450 nm
4. Absorbent paper or paper towel
5. 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.
6. Samples containing sodium azide should not be used in the Assay.

**20x Enzyme Conjugate**
Prepared 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.

Before proceeding with the assay, bring all reagents to room temperature (18–26 °C).

1. Pipette 25 μL of the standards, control, or specimen into the assigned well.
2. Add 100 μL of working Testosterone-enzyme conjugate reagent to all wells (see Preparation Instructions).
3. Swirl the microplate gently for 20–30 seconds to mix the reagents.
4. Cover the plate and incubate for 60 minutes at room temperature.
5. Remove liquid from all wells. Wash wells three times with 300 μL of 1x Wash buffer (see Preparation Instructions). Blot on absorbent paper towels.
6. Add 100 μL of TMB Substrate reagent to all wells.
7. Cover the plate and incubate at room temperature for 15 minutes.
8. Add 50 μL of Stop Solution to each well and gently mix for 15–20 seconds.
9. Read the absorbance on ELISA Reader for each well at 450 nm within 15 minutes after adding the Stop Solution.

It is recommended that serum samples be run in duplicate.

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 ($A_{450}$) 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 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.

Example of the standard curve

<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>

Expected normal values

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following testosterone ranges values were obtained from literature and may be used as initial guideline only:

Males: prepubertal (late), 0.1–0.2 ng/mL
      Adult 3.0–10.0 ng/mL

Females: prepubertal (late), 0.1–0.2 ng/mL
      follicular phase, 0.2–0.8 ng/mL
      luteal phase, 0.2–0.8 ng/mL
      post-menopausal, 0.08–0.35 ng/mL

References

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

©2014 Sigma-Aldrich Co. LLC. All rights reserved. SIGMA-ALDRICH is a trademark of Sigma-Aldrich Co. LLC, registered in the US and other countries. Sigma brand products are sold through Sigma-Aldrich, Inc. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see product information on the Sigma-Aldrich website at www.sigmaaldrich.com and/or on the reverse side of the invoice or packing slip.