Testosterone (Plasma) ELISA

For the quantitative determination of testosterone in human plasma

*Please read carefully due to Critical Changes, e.g., second control added.*

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 11-TESHU-E01-PLAS
Size: 96 wells
Version: 5.0 October 26, 2010 - ALPCO February 22, 2011
**INTENDED USE**
This kit is for the direct quantitative determination of Testosterone by enzyme immunoassay in human plasma. This kit is for research use only. It is not for use in diagnostic procedures.

**PRINCIPLE OF THE TEST**
The principle of the following enzyme immunoassay test follows the typical competitive binding schematic. Competition occurs between an unlabeled antigen (present in standards, controls, and samples) and an enzyme-labeled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stop solution. The absorbance is measured on a microtiter plate reader. The intensity of the color formed is inversely proportional to the concentration of testosterone in the sample. A set of standards is used to plot a standard curve from which the amount of testosterone in samples and controls can be directly read.

**RESEARCH APPLICATIONS**
Testosterone is the most important male sex hormone; it is responsible for genital development, beard growth, muscle development and general male characteristics. The measurement of serum or plasma levels is an index of Leydig cell function and high or low values correlate well with hypo- or hypergonadism. In females small amounts of testosterone are produced by the adrenals and ovaries. High levels of testosterone in females indicates excessive androgen production and are found in progressive hirsutism and virilization, Cushing’s syndrome and a deficiency in one or more of the specific enzymes required for normal steroid biosynthesis.

**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. Control materials or sample pools should be included in every run at high and low levels for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
5. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
6. A calibrator curve must be established for every run.
7. The controls should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing, or improper reagent storage may be indicated when assay values for the controls do not reflect established ranges.
9. When reading the microplate, the presence of bubbles in the microwells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
10. The substrate solution (TMB) is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case the substrate solution should not be used.
11. When dispensing the substrate and stop solution, do not use pipettes in which these liquids will come into contact with any metal parts.
12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
13. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
14. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

LIMITATIONS
1. All the reagents within the kit are calibrated for the direct determination of testosterone in human plasma. The kit is not calibrated for the determination of testosterone in saliva or other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored plasma.
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 samples with high concentrations. The use of any other reagent may lead to false results.
5. The occurrence of heterophilic antibodies in individuals regularly exposed to animals or animal products has the potential of causing interference in immunological tests. Consequently, if false results are suspected, the subject’s background, including the frequency of exposure to animals/products, should be considered.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL
Human serum that may be used in the preparation of the standards and controls has been tested and found to be non-reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. However, no test method can offer complete assurance that HIV, HCV and Hepatitis B virus or any other infectious agents are absent. The reagents should be considered potential biohazards and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS
Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contact is made with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE
Approximately 0.2 ml of plasma is required per duplicate determination. Consider all human specimens as potential biohazards and take appropriate precautions when handling.

SPECIMEN PRETREATMENT
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. Distilled or deionized water
4. Plate shaker
5. Microwell plate reader with a filter set at 450nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10).
**REAGENTS PROVIDED**

1. **Rabbit Anti-Testosterone Antibody Coated Microwell Plate-Break Apart Wells - Ready To Use.**
   Contents: One 96 well (12 x 8) polyclonal antibody-coated microwell plate in a resealable pouch with desiccant.
   Storage: Refrigerate at 2-8°C
   Stability: 12 months or as indicated on label.

2. **Testosterone-Horseradish Peroxidase (HRP) Conjugate Concentrate - Requires Preparation.**
   Contents: Testosterone-HRP conjugate in a protein-based buffer with a non-mercury preservative.
   Volume: 300 μl/vial
   Storage: Refrigerate at 2-8°C
   Stability: 12 months or as indicated on label.
   Preparation: Dilute 1:50 in assay buffer before use (e.g., 40 μl of HRP in 2 ml of assay buffer). If the whole plate is to be used dilute 240 μl of HRP in 12 ml of assay buffer. Discard any that is left over.

3. **Testosterone Calibrators - Ready To Use.**
   Contents: Six vials containing testosterone in a human serum-based buffer with a non-mercury preservative. Prepared by spiking serum with a defined quantity of testosterone.
   *Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>Concentration*</th>
<th>Volume/Vial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator A</td>
<td>0 ng/ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Calibrator B</td>
<td>0.08 ng/ml</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>Calibrator C</td>
<td>0.42 ng/ml</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>Calibrator D</td>
<td>1.67 ng/ml</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>Calibrator E</td>
<td>5.0 ng/ml</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>Calibrator F</td>
<td>16.7 ng/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 label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

4. **Controls - Ready To Use.**
   Contents: Two vials containing testosterone in a human serum-based buffer with a non-mercury preservative. Prepared by spiking serum with a defined quantity of testosterone. Refer to vial labels for expected value and acceptable range.
   Volume: 0.5 ml/vial
   Storage: Refrigerate at 2-8°C
   Stability: 12 months in unopened vial or as indicated on label. Once opened, the controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

5. **Wash Buffer Concentrate - Requires Preparation.**
   Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
   Volume: 50 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 before use. If the whole plate is to be used, dilute 50 ml of the wash buffer concentrate in 450 ml of water.

6. **Assay Buffer (plasma)** - Ready To Use.
Contents: One vial containing a protein-based buffer with a non-mercury preservative.
Volume: 15 ml/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

7. **TMB Substrate** - Ready To Use.
Contents: One bottle containing tetramethylbenzidine 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.

8. **Stop Solution** - Ready To Use.
Contents: One vial containing 1M sulfuric acid.
Volume: 6 ml/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

**ASSAY PROCEDURE**
Specimen Pretreatment: **None**.
All reagents must reach room temperature before use. Calibrators, controls and samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Prepare working solutions of the testosterone-HRP conjugate and wash buffer.
2. Remove the required number of microwell strips. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette 50 μl of each calibrator, control and sample into correspondingly labeled wells in duplicate.
4. Pipette 100 μl of the conjugate working solution into each well (it is recommended to use a multichannel pipette).
5. Incubate on a plate shaker (approximately 200 rpm) for 1 hour at room temperature.
6. Wash the wells 3 times with 300 μl of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry (the use of a washer is recommended).
7. Pipette 150 μl of TMB substrate into each well at timed intervals.
8. Incubate on a plate shaker for 10-15 minutes at room temperature (or until calibrator A attains a dark blue color for desired OD).
9. Pipette 50 μl of stop solution into each well at the same time intervals as in step 7.
10. Read the plate on a microwell plate reader at 450 nm within 20 minutes after addition of the stop solution.*

* 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 sample results.
**CALCULATIONS**

1. Calculate the mean optical density of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4 or 5-parameter curve is recommended.
3. Calculate the mean optical density of each unknown duplicate.
4. Read the values of the unknowns directly off the calibrator curve.
5. If a sample reads more than 20 ng/ml, dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

**TYPICAL TABULATED DATA**

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>OD 1</th>
<th>OD 2</th>
<th>Mean OD</th>
<th>Value (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.391</td>
<td>2.357</td>
<td>2.374</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>2.069</td>
<td>1.942</td>
<td>2.006</td>
<td>0.1</td>
</tr>
<tr>
<td>C</td>
<td>1.533</td>
<td>1.578</td>
<td>1.556</td>
<td>0.5</td>
</tr>
<tr>
<td>D</td>
<td>0.984</td>
<td>1.039</td>
<td>1.012</td>
<td>2</td>
</tr>
<tr>
<td>E</td>
<td>0.606</td>
<td>0.575</td>
<td>0.591</td>
<td>6</td>
</tr>
<tr>
<td>F</td>
<td>0.290</td>
<td>0.293</td>
<td>0.292</td>
<td>20</td>
</tr>
<tr>
<td>Unknown</td>
<td>1.266</td>
<td>1.238</td>
<td>1.252</td>
<td>1.1</td>
</tr>
</tbody>
</table>

**TYPICAL CALIBRATOR CURVE**

Sample curve only. Do not use to calculate results.

**REFERENCES**

12. Palatsi, R., et al., Serum total and unbound testosterone and SHBG in female acne patients treated with two different oral contraceptives ACTA DERMATOVENEREOL 64:51 7, 1984