Testosterone ELISA Kit

Prod. No.: DEIA285
Pkg. Size: 96T

INTENDED USE
Enzyme Immunoassay for the Quantitative Determination of Testosterone in Plasma and Serum

GENERAL DESCRIPTION
The steroid hormone testosterone belongs to the androgens, which are produced in the testicles, adrenal cortex, and in small quantities also in the ovary. Besides testosterone, androstenedione and androsterone are of importance in this group. The androgens stimulate the development of male sexual organs as well as the differentiation of the secondary male sex characteristics. Steroid hormones are used since some decades to enhance the growth of cattle and other animals. Hormones employed can be endogenous ones (testosterone, progesterone, estradiol) or synthetic products. Since the hormones show side-effects when ingested with the meat, e.g. genotoxic, neurobiologic and carcinogenic effects, the risks for the consuming persons should be estimated. In contrast to the USA, where the use of sex hormones as implants is allowed, in the European Union all substances, which lead to an enhancement of natural growth, are forbidden (88/146/EEC and 96/22/EC). These guidelines imply an obligation to monitor these hormones and their metabolites in animals and animal products. Control methods like special gaschromatographic procedures have been established. The ELISA belongs to the most sensitive procedures and can detect residues down to 0.1 ng/mL (100 ppt).

PRINCIPLE OF THE TEST
The Testosterone quantitative test is based on the principle of the enzyme linked immunosorbent assay. An antibody directed against testosterone is bound on the surface of a microtiter plate. Testosterone containing samples or standards and a testosterone-peroxidase conjugate are given into the wells of the microtiter plate. Enzyme labeled and free testosterone compete for the antibody binding sites. After a one hour incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A substrate solution is added and incubated for 10 minutes, resulting in the development of a blue colour. The colour development is inhibited by the addition of a stop solution, and the colour turns yellow. The yellow colour is measured photometrically at 450 nm. The concentration of testosterone is indirectly proportional to the colour intensity of the test sample.

REAGENTS PROVIDED
The kit contains reagents for 96 determinations. They have to be stored at 2-8°C. Expiry data are found on the labels of the bottles and the outer package.
1. Microtiter plate consisting of 12 strips with 8 breakable wells each, coated with antitestosterone.
2. Testosterone Standards (0; 100; 500; 1000; 5000; 15000 pg/mL): 1.0 mL each, dyed red, ready-to-use.
3. Conjugate (Testosterone-Peroxidase): 100 μL as 100x concentrate, dyed red. Dilute 1:100 with conjugate diluent.
4. Conjugate Diluent: 6 mL, dyed red, ready-to-use.
5. Substrate Solution (TMB): 15 mL; ready-to-use.
6. Stop Solution (0.5 M H₂SO₄): 15 mL; ready-to-use.
7. Sample Diluent (PBS): 2 x 50 mL; dyed red, ready-to-use.
8. Washing Solution (PBS + Tween 20): 30 mL as 10x concentrate, dyed blue. Dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.
9. Two plastic foils to cover the strips during the incubation.
10. Plastic bag to store unused microtiter strips.

MATERIALS REQUIRED BUT NOT PROVIDED

Instrumentation
1. 10, 50, 100 and 1000 μL-micropipets
2. Microtiter plate shaker
3. ELISA reader (450 nm)
4. Freezer (-25/-60°C)

Reagents
1. TBME (tertiary butyl-methyl-ether)
2. Petrol ether (boiling range 30-50°C)

ASSAY PROCEDURE

1. Reagent And Sample Preparation
1) Serum/Plasma
To 1 mL plasma or serum 5 mL ether mixture (TBME/petrol ether 30/70 v/v, boiling range 30-50°C) is added in a glass vial. The vial is heavily shaken for 20 minutes. Afterwards the mixture is put into a freezer for 60 minutes at -25°C (or 30 minutes at -60°C) and the supernatant is decanted. The supernatant is evaporated to dryness at 60°C in a water bath. The residue is reconstituted by adding 1.0 mL sample diluent. It is heavily shaken for 1 minute and warmed to 37°C for 5 minutes. The last step is repeated two more times.
2. Assay Steps
1) Prepare samples as described above.
2) Dilute the conjugate concentrate 1+100 with conjugate diluent (e.g. 10 μL conjugate + 1000 μL diluent).
3) Pipet 100 μL standards or prepared samples in duplicate into the appropriate wells of the microtiter plate. Immediately add 50 μL diluted testosterone- peroxidase conjugate into each well.
4) Cover the microtiter plate with a plastic foil and incubate for 60 minutes at room temperature on a microtiter plate shaker (or 90 minutes without shaker).
5) Wash the plate three times as follows: Discard the contents of the wells (dump or aspirate). Pipet 300 μL of diluted washing solution into each well. After the third repetition empty the wells again and remove residual liquid by striking the plate against a paper towel. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbencies.
6) Pipet 100 μL of substrate solution into each well.
7) Allow the reaction to develop in the dark (e.g. cupboard or drawer; the chromogen is light-sensitive) for 10 minutes at room temperature.
8) Stop enzyme reaction by adding 100 μL of stop solution (0.5 M H2SO4) into each well. The blue colour will turn yellow upon addition.
9) After thorough mixing, measure absorbance at 450 nm (reference wavelength 620 nm), using an ELISA reader. The colour is stable for 30 minutes.

REFERENCES

EVALUATION & CALCULATION
1. Evaluation
The following table contains an example for a typical standard curve. The binding is calculated as percent of the absorption of the 0 pg/mL standard. These values are only an example and should not be used instead of the standard curve which has to be measured in every new test.

<table>
<thead>
<tr>
<th>Testosterone (pg/mL)</th>
<th>(% binding of 0 pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>100</td>
<td>89</td>
</tr>
<tr>
<td>500</td>
<td>64</td>
</tr>
<tr>
<td>1000</td>
<td>44</td>
</tr>
<tr>
<td>5000</td>
<td>7</td>
</tr>
<tr>
<td>15000</td>
<td>2</td>
</tr>
</tbody>
</table>

2. Calculation
1) Calculate the average optical density (OD 450 nm) for each set of reference standards or samples.
2) Construct a standard curve by plotting the mean optical density obtained for each reference standard against its concentration in pg/mL on semilog graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis.
3) Using the mean optical density value for each sample, determine the corresponding concentration of testosterone in pg/mL from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4) The diluted samples must be further converted by the appropriate dilution factor (1.0 for the above described extraction). The factor is dependent on the sample preparation procedure employed.

ASSAY CHARACTERISTICS

Sensitivity: The sensitivity of the Testosterone test is 100 pg/mL (based on the standard curve).

Recovery: The recovery of spiked samples was determined to 90% for plasma.

Intra-assay Precision: The intra-assay variation of the testosterone test was determined to 3%.

Cross-reactivity relative to testosterone (=100%)

<table>
<thead>
<tr>
<th>Nor-Testosterone</th>
<th>&lt;10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticosterone</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>&lt;0.5%</td>
</tr>
<tr>
<td>Progesterone</td>
<td>&lt;0.5%</td>
</tr>
</tbody>
</table>