



Test Instruction

Testosterone

96 Tests

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

Cat.-No.: TES-E01

Version: January 21st, 2010

Sensitivity	100 pg/mL
Recovery (spiked samples)	90%
Incubation Time	70 min

General Information

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 implantates 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 **Immunolab 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.

Precautions

Full compliance of the following good laboratory practices (GLP) will determine the reliability of the results:

1. Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
4. Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.
5. Use a separate disposable tip for each specimen to prevent cross-contamination.
6. All specimens and standards should be run at the same time, so that all conditions of testing are the same.
7. Do not mix components from different batches.
8. Do not use reagents after expiration date.
9. Check both precision and accuracy of the laboratory equipment used during the procedure (micropipets, ELISA reader etc.).

Health and safety instructions

1. Do not smoke or eat or drink or pipet by mouth in the laboratory.
2. Wear disposable gloves whenever handling patient specimens.
3. Avoid contact of substrate and stop solution with skin and mucosa (possible irritation, burn or toxicity hazard). In case of contact, rinse the affected zone with plenty of water.
4. Handling and disposal of chemical products must be done according to good laboratory practices (GLP).

Reagents

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 anti-testosterone.
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. The diluted conjugate is not stable and cannot be stored.
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.
11. Instruction Manual.

Additional Instrumentation and Reagents (not provided)

Instrumentation

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

Reagents

- TBME (tertiary butyl-methyl-ether)
- Petrol ether (boiling range 30-50°C)

Sample Preparation

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.

Procedure

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.

Calculation of results

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 semi-log 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.

Typical Standard Values

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.

Testosterone (pg/mL) (% binding of 0 pg/mL)

0	100
100	89
500	64
1000	44
5000	7
15000	2

Performance

Sensitivity

The sensitivity of the **Immunolab 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%)

Nor-Testosterone	< 10%
Corticosterone	< 1%
17 β -Estradiol	< 0,5%
Progesterone	< 0,5%

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