TESTOSTERONE

ENZYME IMMUNOASSAY TEST KIT
Catalog Number: 10007

Enzyme Immunoassay for the Quantitative Determination of TESTOSTERONE in Human Serum or plasma

INTRODUCTION OF IMMUNOASSAY

Testosterone (17β-hydroxyandrost-4-ene-3-one) is a C19 steroid with an unsaturated bond between C-4 and C-5, a ketone group in 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 ca. 50% of circulating testosterone is derived from peripheral conversion of androstenedione, ca. 25% from the ovary and ca. 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 men, 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.

The Testosterone EIA kits are designed for the measurement of total Testosterone in human serum.

PRINCIPLE OF THE TEST

The Testosterone EIA is based on the principle of competitive binding between Testosterone in the test specimen and Testosterone-HRP conjugate for a constant amount of rabbit anti-Testosterone. In the incubation, goat anti-rabbit IgG-coated wells are incubated with 10 µL of Testosterone standards, controls, patient samples,100 µL Testosterone-HRP conjugate reagent and 50 µL rabbit anti-Testosterone reagent at 37°C for 90 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 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of 2N HCl, and the absorbance is measured spectrophotometrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled Testosterone in the sample. A standard curve is obtained by plotting the concentration of the standard versus the absorbance. The Testosterone concentration of the specimens and controls run concurrently with the standards can be calculated from the standard curve.

MATERIALS AND COMPONENTS

Materials Provided with Test Kit

1.Goat Anti-Rabbit IgG-coated microtiter wells, 96 wells
2.Testosterone Reference Standards: 0, 0.1, 0.5, 2.0, 6.0 and 18.0 ng/mL. Liquids, 0.5 mL each, ready to use.
3.Rabbit Anti-Testosterone Reagent 7 mL
4.Testosterone-HRP Conjugate Reagent , 12 mL
5.TMB Substrate,12 mL.
6.Stop Solution, 12mL.
7.Wash Buffer Concentrate(50X), 15mL

Materials required but not provided:

1.Precision pipettes: 10 µL, 50 µL, 100 µL, and 1.0 mL.
2.Disposable pipette tips.
3.Distilled or deionized water.
4.Vortex mixer or equivalent.
5.Absorbent paper or paper towel.
6.Linear-linear graph paper.
7.Microtiter plate reader.

SPECIMEN COLLECTION AND PREPARATION

1.Serum or EDTA plasma should be used. No special pretreatment of sample is necessary.

2.Serum or plasma samples may be stored at 2-8°C for up to 24 hours, and should be frozen at −10°C or lower for longer periods. Do not use grossly hemolyzed or grossly lipemic specimens.

3.Please note: Samples containing sodium azide should not be used in the assay.

STORAGE OF TEST KIT AND INSTRUMENTATION

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 O.D. at 450 nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

- All reagents should be brought to room temperature (18-22°C) before use.
- Samples with expected Testosterone concentrations over 18 ng/mL may be quantitated by dilution with diluent available from the company.
- Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 mL of Wash Buffer (50x) into distilled water to prepare 750 mL of washing buffer (1x). Mix well before use.

ASSAY PROCEDURE

1.Secure the desired number of coated wells in the holder.

2.Dispense 10 µL of standards, specimens and controls into appropriate wells.

3.Dispense 50 µL of rabbit anti-Testosterone reagent to each well.

4.Thoroughly mix for 30 seconds. It is very important to mix them completely.

5.Dispense 100 µL of Testosterone-HRP Conjugate Reagent into each well.

6.Incubate at 37°C for 90 minutes.

7.Rinse and flick the microwells 5 times with washing Buffer (1X).

8.Dispense 100 µL of TMB Substrate to each well. Gently mix for 10 seconds.

9.Incubate at room temperature (18-22°C) for 20 minutes.

10.Stop the reaction by adding 100µL of Stop Solution to each well.

11.Gently mix 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.

12.Read absorbance at 450 nm with a microtiter well reader within 15 minutes.

IMPORTANT NOTE

1.The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

2.If there are bobbles existing in the wells, the false readings will be created. Please use distilled water to remove the bobbles before adding the substrate.

CALCULATION OF 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.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against Testosterone concentrations shown in the X axis. Note: This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each laboratory must provide its own data and standard curve in each experiment.

TESTOSTERONE (ng/mL)	Absorbance (450nm)
0.0	3.096
0.1	2.700
0.5	2.185
2.0	1.709
6.0	1.105
18.0	0.516

EXPECTED VALUES AND SENSITIVITY

Each laboratory should establish its own normal range based on the patient population. The Testosterone EIA was performed on randomly selected outpatient clinical laboratory samples. The results of these determinations are as follows:

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

The minimum detectable concentration of the Testosterone ELISA assay as measured by 2 SD from the mean of a zero standard is estimated to be 0.05 ng/mL.

CLINICAL APPLICATION

In Male:

In man, the determination of testosterone is used as an indicator for the function of the testes: low hormone levels are found in cases with Klinefelter's syndrome, cryptorchism or anorchia. Male with testosterone deficiency often present with a number of symptoms such as decreased libido, as well as decreased muscle strength, gynecomastia and infertility.

In Female:

1.Virilizing Disorders:
Testosterone measurements are frequently utilized in the evaluation of virilizing disorders. Testosterone concentrations >2.0 ng/mL may indicate androgen secreting ovarian or adrenal neoplasms.

2.Monitoring of Androgen Suppressing Drugs:
Testosterone measurements may be utilized in women for the adjustment of androgen suppressing drugs and their dosages.

3.Pregnancy:
Testosterone concentrations are relatively consistent during the Pregnancy.

LIMITATIONS OF THE PROCEDURE

1.Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.

2.The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

3.Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

4.The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

REFERENCES

1.Chen, A., Bookstein, J.J., Meldrum, D.R., Diagnosis of a testosterone-secreting adrenal adenoma by selective venous catheterization. **Fertil. Steril**., 1991; 55: 1202-1203.

2.Granoff, A.B. and Abraham, G.E., Peripheral and adrenal venous levels of steroids in a patient with virilizing adrenal adenoma. **Obstet. Gynecol**., 1979; 53:111-115.

3.Bricaire, C., Raynaud, A., Benotmane, A., et al., Selective venous catheterization in the evaluation of hyperandrogenism. **J. Endocrinol Invest**., 1991; 14: 949-956.

4.Heinonen, P.K., Androgen production by epithelial ovarian tumours in post-menopausal women. **Maturitas**, 1991; 13: 117-117-122.

5.Tietz, N.W. ed., **Clinical Guide to Laboratory Tests**, 3rd Edition, W.B. Saunders, Co., Philadelphia, 1995: 578-580.

6.**USA Center for Disease Control/National Institute of Health Manual**, “Biosafety in Microbiological and Biomedical Laboratories"” 1984.

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Chemux Bioscience,Inc.

Website: www.chemux.com

385 Oyster Point Blvd Suite5-6.,South San Francisco,CA94080
Tel:+1-650-872-1800