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

IVD For *in vitro* diagnostic and professional use only

Store at (2° to 8°C)

96 Tests

INTENDED USE
For the Quantitative Determination of Testosterone in Human Serum or plasma.

INTRODUCTION
Testosterone is the primary male sex hormone and an anabolic steroid. In male humans, testosterone plays a key role in the development of male reproductive tissues such as testes and prostate, as well as promoting secondary sexual characteristics such as increased muscle and bone mass, and the growth of body hair. In addition, testosterone is involved in health and well-being, and the prevention of osteoporosis. Insufficient levels of testosterone in men may lead to abnormalities including frailty and bone loss.

Testosterone is a steroid from the androstane class containing a keto and hydroxyl groups at the three and seventeen positions respectively. It is biosynthesized in several steps from cholesterol and is converted in the liver to inactive metabolites. It exerts its action through binding to and activation of the androgen receptor. In humans and most other vertebrates, testosterone is secreted primarily by the testicles of males and, to a lesser extent, the ovaries of females. On average, in adult males, levels of testosterone are about 7 to 8 times as great as in adult females. As the metabolism of testosterone in males is more pronounced, the daily production is about 20 times greater in men. Females are also more sensitive to the hormone.

PRINCIPLE
The Testosterone ELISA 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 25 µl of Testosterone standards, controls, patient samples, 100µl Testosterone-HRP conjugate reagent and 50 µl rabbit anti-Testosterone reagent at 37°C 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 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
Materials Provided

1. Goat Anti-Rabbit IgG-coated microtiter wells, 96 wells.
2. Testosterone Reference Standards: 0 (white cap vial), 0.1 (yellow cap vial), 0.5 (green cap vial), 2.0 (blue cap vial), 6.0 (purple cap vial) and 18.0 (red cap vial) ng/ml. Liquids, 0.5 ml each, ready to use.
3. Rabbit Anti-Testosterone Reagent (7 ml orange liquid colored with transparent cap bottle).
4. Testosterone-HRP Conjugate Reagent, 12 ml (Blue colored reagent in transparent cap bottle).
5. TMB Substrate, 12 ml (brown cap bottle).
6. Stop Solution, 12ml (transparent cap bottle).
7. Wash Buffer Concentrate (50X), 15ml (Green cap bottle).
8. Package insert.

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. Collect serum or plasma samples in accordance with correct medical practices.
2. Cap and store the samples at 2-8°C for no more than 24 hours, and should be frozen at −10°C or lower for longer periods.
3. Serum or EDTA plasma should be used. No special pretreatment of sample is necessary.
4. Avoid grossly hemolytic, lipemic or turbid samples.
5. Please note: Samples containing sodium azide should not be used in the assay.

STORAGE OF TEST KIT AND INSTRUMENTATION
1. Components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8°C.
2. Place unused wells in the sealed bag with desiccants and return to 2-8 °C, under which conditions the wells will remain stable until the labeled expiry date printed.
3. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2.5 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. Remove unused strips from the microwell plate, and store in the original resealable pouch at 2-8°C.
2. To appropriate wells, add 25 µl of standards, specimens and controls
3. To each well, add 50 µl of rabbit anti-Testosterone reagent.
4. Mix thoroughly for 30 seconds. It is very important to mix them completely.
5. To each well, add 100 µl of Testosterone-HRP Conjugate Reagent.
6. Incubate at 37°C for 60 minutes.
7. Rinse and flick the microwells 5 times with washing Buffer (1X).
8. To each well, add 100 µl of TMB Substrate .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 (450 nm)
0.0	2.295
0.1	1.905
0.5	1.453
2.0	0.798
6.0	0.482
18.0	0.200

EXPECTED VALUES AND SENSITIVITY

Each laboratory should establish its own normal range based on the patient population. The Testosterone ELISA 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. For professional use only
2. 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.
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.
5. Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot gurantee it will eliminate all the effects of that.

REFERENCES

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REF	Catalogue Number		Temperature limit
IVD	In Vitro diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry