FREE TESTOSTERONE

INTEND USE
This kit is for the direct quantitative determination of free testosterone by enzyme immunoassay in human serum or plasma.

INTRODUCTION OF Free Testosterone
Testosterone (17β-hydroxyandrost-4-ene-3-one) is a C-19 steroid secreted from the testis and the adrenal cortex in men and from the adrenal cortex and ovaries in women. Testosterone is the most important androgen secreted into the blood. Testosterone measurements are used mainly for clinical evaluation of hypogonadism in males and hyperandrogenic states in females. 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. 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.
Testosterone circulates in the blood bound to three proteins: sex hormone binding globulin (60~80%), while SHBG effectively inhibits testosterone action. Only about 1~2% of the total circulating testosterone remains unbound or free. Even though it is still under investigation, most researchers accept the free testosterone determination as a measure of the biologically active fraction. Free testosterone determinations and recommended to overcome the influences caused by variations in transport proteins on the total testosterone concentration.
Measurement of the free or unbound fraction of serum testosterone has been proposed as a means of estimating the physiologically bioactive hormone. Free testosterone levels are elevated in women with hyperandrogenism associated with hirsuitism in the presence or absence of polycystic ovarian disease. In addition, free testosterone measurements may be more useful than total testosterone in stituations where SHBG is increased or decreased (e.g. hypothyroidism and obesity).
The Free Testosterone EIA kits are designed for the measurement of Free Testosterone in human serum.

PRINCIPLE OF THE TEST
The Free Testosterone EIA is based on the principle of competitive binding between Free Testosterone in the test specimen and Free Testosterone-HRP conjugate for a constant amount of rabbit anti-Free Testosterone. In the incubation, goat anti-rabbit IgG-coated wells are incubated with 20 µl of Free Testosterone standards, controls, patient samples, 100 µl Free Testosterone-HRP conjugate reagent and 50 µl rabbit anti-Free Testosterone reagent at 37°C for 60 minutes.
During the incubation, a fixed amount of HRP-labeled Free Testosterone competes with the endogenous Free Testosterone in the standard, sample, or quality control serum for a fixed number of binding sites of the specific Free Testosterone antibody. Thus, the amount of Free Testosterone peroxidase conjugates immunologically bound to the well progressively decreases as the concentration of Free Testosterone in the specimen increases.
Unbound Free 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 1N 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 Free 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 (6 doses): 0, 0.2, 2.0, 8.0, 30 and 60 pg/ml. Liquids, 0.5 ml each, ready to use. For details see labels.
3. Rabbit Anti-Free Testosterone Reagent. 7 ml
4. Free Testosterone-HRP Conjugate Reagent, 12 ml
5. TMB Substrate,12 ml.
6. Stop Solution, 12ml.
7. Wash Buffer Concentrate(50X), 15ml
8. Control Set. 0.5 ml each

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 Free Testosterone concentrations over 60 pg/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.

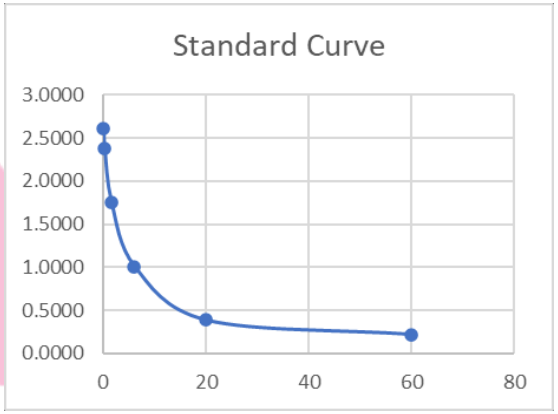
ASSAY PROCEDURE
1. Secure the desired number of coated wells in the holder.
2. Dispense 20 µl of standards, specimens and controls into appropriate wells.
3. Dispense 50 µl of rabbit anti-Testosterone reagent to each well.
4. Thoroughly mix for 30 seconds. It is very important to mix them completely.
5. Dispense 100 µl of Testosterone-HRP Conjugate Reagent into each well.
6. Incubate at 37°C for 60 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 pg/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 Free Testosterone in pg/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 Free 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.

Free Testosterone (pg/ml)	Free Testosterone (pg/ml)
0.0	2.609
0.2	2.379
2.0	1.755
8.0	1.010
30.0	0.388
60.0	0.185



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EXPECTED VALUES AND SENSITIVITY

Each laboratory should establish its own normal range based on the patient population. The Free Testosterone EIA was performed on randomly selected outpatient clinical laboratory samples. The results of these determinations are as follows table 1:

Table 1:

Population (Gender / Age)	Free Testosterone Range (pg/ml)
Male / 20 – 39	9.2 – 34.6
Male / 40 – 59	6.1 – 30.3
Male / ≥ 60	6.1 – 27.9
Female 20 – 39	0.2 – 6.1
Female 40 – 59	0.3 – 4.4
Female ≥ 60	0.5 – 3.4

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

PERFORMANCE CHARACTERISTICS

1) Limit of Blank

The Limit of Blank (LOB) was calculated by measuring the blank several times and calculating 95th percentile of the distribution of the test values on truly blank samples deviate significantly from blank measurements. The LOB of this assay is estimated to be 0.038 pg/ml.

2) Limit of Detection

The minimum detectable concentration is 0.085 pg/ml. The sensitivity is definied as the mean plus two standard deviations of 20 replicated of the zero calibrator. The sensitivity is 0.02 pg/ml.

3) Detection Range

Limit of Quantitation (LOQ) is the lowest concentration at which the analytz cannot only be reliably detected but at which some predefined goal of bias and imprecision are met. The Detection Range is from 0.12 to 60 pg/ml without need of sample dilution.

4) Precision Study

Three concentration levels of Free Testosterone samples were used to determine the intra & inter precision of Free Testosterone ELISA assay kit. The results were shown in the table 2.

Table 2

Intra Precision Study				
Concentrations	**NO**	**Mean (pg/ml)**	**S.D.**	**CV**
Level 1	16	2.86	0.13	4.38
Level 2	16	24.21	1.53	6.33
Level 3	16	43.53	0.81	1.86

Inter Precision Study				
Concentrations	**NO**	**Mean (pg/ml)**	**S.D.**	**CV**
Level 1	16	2.17	0.13	6.20
Level 2	16	15.16	0.81	5.33
Level 3	16	40.33	2.57	6.38

5) Accuracy

Run total 63 samples from Blood Bank, including 33 female samples (age from 19~63 years old); 30 male samples (age from 20~64 years old). The reference range as table 1 shown above. The accuracy is greater than 90%.

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.
5. It is important that the time of reaction in each well is held constant to achieve reproducible results.
6. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
7. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers, and/or the automated instruments used with this device, and to perform routine preventative maintenance.

REFERENCES

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

CONT. 96 TEST CODIGO: RSET014