

**CALCULATION OF RESULTS**

1. Calculate the average absorbance values for each set of standards, controls and patient samples
2. Construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration in pg/ml with absorbance value on the vertical(Y) axis and concentration on the horizontal (X) axis
3. Using the mean absorbance value for each sample determine the corresponding concentration of Free Testosterone from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. Automated method: Computer programs using cubic spline, 4 PL (4 Parameter Logistics) or Logit-Log can generally give a good fit.
5. The concentration of the samples can be read directly from this standard curve. Samples with Free Testosterone concentration higher than the concentration of the highest standard have to be diluted with zero standard. For the calculation of the concentrations this dilution factor has to be taken into account.

**Example of a standard Curve**

	OD 450 nm	Conc. pg/mL
Std 1	2.762	0
Std 2	1.528	0.15
Std 3	0.903	1.5
Std 4	0.468	8
Std 5	0.140	25
Std 6	0.075	60

**LIMITATION OF THE TEST**

1. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

**REFERENCES**

1. McCann D, Kirkish L. Evaluation of Free Testosterone in serum. J.Clin. Immunoassay 1985; 8:234-236.
2. Ekins R.P. Free hormones in blood J. Clin. Immunoassay 1984; 7(2): 163-180.
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4. Odland V. et al. Plasma androgenic activity in women with acne vulgaris and in healthy girls before, during and after puberty. Clin.Endocrinology 1982; 16:243-249.
5. Green P.J. Free Testosterone determination by ultrafiltration and comparison with dialysis.Clin.Chem. 1982;28:163-180.
6. Wu Ch. Plasma free and protein-bound testosterone in hirsutism. Obstet.Gynecol 1982; 60:188-194.
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Cat#: FT178S (96 Tests)

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[www.calbiotech.com](http://www.calbiotech.com)**Free Testosterone ELISA**

Catalog No. FT178S (96 Tests)

**INTENDED USE**

The Calbiotech, Inc. (CBI) Free Testosterone ELISA Kit is intended for the measurement of Free Testosterone in serum or plasma.

**SUMMARY AND EXPLANATION**

Testosterone is a steroid hormone from the androgen group. Testosterone is primarily secreted in the testes of males and the ovaries of females although small amounts are secreted by the adrenal glands. Testosterone is the principal male sex hormone, is an anabolic steroid and, for both males and females, plays a key role in health and well-being. Testosterone within the circulation is principally bound to proteins, the most important of which, is sex hormone binding globulin (SHBG). Measurement of the free or unbound fraction of serum testosterone has been proposed as a mean of estimating the physiologically bioactive hormone. Free testosterone levels are elevated in women with hyperandrogenism associated with hirsutism in the presence or absence of polycystic ovarian disease. In addition, free testosterone measurements may be more useful than total testosterone in situations where SHBG is increased or decreased (e.g. hypothyroidism and obesity).

**PRINCIPLE OF THE TEST**

The CBI Testosterone ELISA kit 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-Free Testosterone. In the incubation, goat anti-rabbit IgG-coated wells are incubated with 25µl of Testosterone standards, patient samples, 50µl testosterone-HRP conjugate reagent and 50µl rabbit anti-free testosterone reagent at room temperature for 60 minutes. During the incubation, a fixed amount of HRP labeled testosterone competes with the endogenous testosterone in the standard and sample, for a fixed number of binding sites of the specific free testosterone antibody. Thus, the amount of testosterone peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of free testosterone in the specimen increases. Unbound testosterone peroxidase conjugate is then removed and the wells washed. Next, 100µl of TMB Reagent is added and incubated at room temperature for 15 minutes, resulting in the development of blue color. The color development is stopped with the addition of 50µl stop solution, and the absorbance is measured spectrophotometrically at 450nm. A standard curve is prepared relating color intensity to the concentration of the Free Testosterone.

MATERIALS PROVIDED	96 Tests
1. Microwells coated with Goat anti-rabbit IgG	12x8x1
2. Standard: 6 vials (ready to use)	0.5 ml
3. Enzyme Conjugate (ready to use)	7 ml
4. Rabbit Anti-Testosterone Reagent (ready to use)	7 ml
5. TMB substrate (ready to use)	12 ml
6. Stop solution (ready to use)	12 ml
7. Wash Solution 20x Concentrated	25ml

#### MATERIAL NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

#### STORAGE AND STABILITY OF THE KIT

1. Store the kit at 2 – 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. Opened standards are stable for 6 months at 2 – 8° C all other reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun, or strong light.

#### WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. For laboratory use.
3. Potential biohazardous materials:  
The standards contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
4. This kit is designed for research use only.
5. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.
6. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed. It is recommended that serum samples be run in duplicate.
7. This test kit is designed for Research and Development use only.
8. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.

#### SPECIMEN COLLECTION HANDLING

1. Serum: Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature.
2. Plasma: Whole blood should be collected into centrifuge tubes containing anti-coagulant and centrifuged immediately after collection.
3. Do not use haemolytic, icteric or lipaemic serum.
4. Testosterone can be determined in plasma as well as in serum of patients who have been fasting. The clinical significance of the determination of Free Testosterone can be invalidated if the patient was treated with cortisone or natural or synthetic steroids
5. Specimens which are not used at the same day of collection have to be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing
6. Samples with values greater than the highest standard should be diluted with standard 0 and reassayed.

#### PREPARATION OF REAGENTS

**20X Wash Buffer:** Prepare 1X Wash Buffer by adding the contents of the bottle (25ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

#### ASSAY PROCEDURE

All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming. Once the test has been started, all steps should be completed without interruption.

1. Secure the desired number of microwells strips in the holder.
2. Dispense 25 µl Testosterone Standards, controls and samples with new disposable tips into appropriate wells.
3. Dispense 50ul anti-testosterone reagent into each well.
4. Dispense 50µl Enzyme Conjugate into each well.
5. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
6. Incubate for 1 hour at room temperature.
7. Briskly shake out the contents of the wells. Rinse the wells 3 times with diluted wash solution. Strike the wells sharply on absorbent paper to remove residual water droplets.  
**NOTE:** The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
8. Add 100 µl of Substrate Solution to each well.
9. Incubate for 15 minutes at room temperature in the dark.
10. Stop the enzymatic reaction by adding 50 µl of Stop Solution into each well.
11. Read absorbance on ELISA Reader at 450 nm within 10 minutes after adding the stop solution.