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CALBIOTECH

# Free Testosterone ELISA

Catalog No.: FT178S (96 Tests)

## INTENDED USE

The Calbiotech, Inc. Free Testosterone ELISA Kit is intended for the measurement of Free Testosterone in serum or plasma. **For Research Use Only. For professional use only. Not for use in diagnostic procedures.**

## 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. This product measures the unbound biologically active fraction of testosterone. Used in research on androgen signaling, steroid hormone bioavailability, and endocrine regulation of reproductive and metabolic physiology.

## PRINCIPLE OF THE TEST

The 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. | Control: 2 vials (ready to use) | 0.5 ml |
| 4. | Enzyme Conjugate (ready to use) | 7 ml |
| 5. | Rabbit Anti-Testosterone Reagent (ready to use) | 7 ml |
| 6. | TMB substrate (ready to use) | 12 ml |
| 7. | Stop solution (ready to use) | 12 ml |
| 8. | Wash Concentrate 20X, 1 bottle | 25 mL |

## MATERIALS NOT PROVIDED

1. Precision pipettes
2. Disposable pipette tips
3. ELISA reader capable of reading absorbance at 450nm
4. Flat-head Vortex mixer
5. Plate shaker
6. Test tubes for sample preparation

**WARNINGS AND PRECAUTIONS**

Potential biohazardous materials:

1. 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.
2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. It is recommended that standards, control and serum samples be run in duplicate
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.

**SPECIMEN COLLECTION AND HANDLING**

This assay is designed for use with human serum or plasma samples obtained in compliance with applicable laws, regulations, and institutional policies. Handle and store samples using procedures appropriate for research use. Samples may be stored refrigerated (2–8 °C) for up to seven days, or frozen (–20 °C or below) for up to six months. Avoid repetitive freeze–thaw cycles.

**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.

**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.