

Free TESTOSTERONE ELISA

40-056-205042
FOR RESEARCH USE ONLY

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1 INTRODUCTION

The Free Testosterone ELISA is an enzyme immunoassay for the quantitative *research* measurement of Free Testosterone in serum and plasma

2 SIGNIFICANCE

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. It is the principal male sex hormone and an anabolic steroid. In both males and females, it plays key roles in health and well-being.

Only 1-2% of circulating testosterone exists as unbound or free testosterone. The majority, approximately 60%, is bound to SHBG with high affinity, while the remainder is loosely bound to albumin. Both the albumin-bound and free fractions may be biologically active, while SHBG effectively inhibits testosterone action.

Testosterone effects can be classified as virilizing and anabolic effects. Anabolic effects include growth of muscle mass and strength, increased bone density and strength, and stimulation of linear growth and bone maturation. Virilizing effects include maturation of the sex organs.

Testosterone levels decline gradually with age in men.

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 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).

3 PRINCIPLE OF THE TEST

The Free Testosterone ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding.

The microtiter wells are coated with an antibody directed towards an antigenic site on the Testosterone molecule. Endogenous Free Testosterone of a sample competes with a Testosterone-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off.

The amount of bound peroxidase conjugate is inversely proportional to the concentration of Free Testosterone in the sample. After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of Free Testosterone in the sample.

Testosterone in the blood is bound to SHBG (60 %) and in lower quantity to other protein. Only the measurement of Free Testosterone (< 1% of Total Testosterone) permits the estimating of the hormone biologically active.

4 PRECAUTION

- This kit is for research use only.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
- All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- Avoid contact with *Stop Solution* containing 0.15 mol/L H₂SO₄. It may cause skin irritation and burns.
- Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.

5 KIT COMPONENTS

5.1 Contents of the Kit

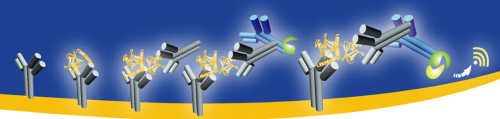
1.


CAL	N
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 N=1 to 5
5 vials, 1 mL each
See exact values on the vial labels
2.

CAL	0
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 1 vial
1 mL



- | | | | | |
|-------|---|--|------|--|
| 3. | <table border="1" style="border-collapse: collapse;"> <tr> <td style="padding: 2px;">Ag</td> <td style="padding: 2px;">HRP</td> </tr> </table> | Ag | HRP | 1 vial, 22 mL, ready to use;
Testosterone conjugated to horseradish peroxidase |
| Ag | HRP | | | |
| 4. |  | Microtiterwells, 12x8 (break apart) strips, 96 wells;
Wells coated with a anti-Testosterone antibody. | | |
| 5. | <table border="1" style="border-collapse: collapse;"> <tr> <td style="padding: 2px;">CHROM</td> <td style="padding: 2px;">TMB</td> </tr> </table> | CHROM | TMB | 1 vial, 12 mL, ready to use;
H ₂ O ₂ /TMB 0.25g/L. Avoid any skin contact |
| CHROM | TMB | | | |
| 6. | <table border="1" style="border-collapse: collapse;"> <tr> <td style="padding: 2px;">STOP</td> <td style="padding: 2px;">SOLN</td> </tr> </table> | STOP | SOLN | 1 vial, 12 mL, ready to use;
Contains 0.15M H ₂ SO ₄ . Avoid contact with the stop solution. It may cause skin irritations and burns. |
| STOP | SOLN | | | |

5.1.1 Equipment and material required but not provided

- o A microtiter plate calibrated reader (450 ± 10 nm)
- o Calibrated variable precision micropipettes.
- o Absorbent paper.
- o Distilled water
- o Incubator 37°C

5.2 Storage and stability of the Kit

When stored at 2-8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2-8°C. Microtiter wells must be stored at 2-8°C. Once the foil bag has been opened, care should be taken to close it tightly again.

5.3 Preparation of Reagents

Allow all reagents and required number of strips to reach room temperature prior to use.

Calibrators

Before use, mix for 5 min. with rotating mixer

Note: The opened calibrators are stable for 6 months at 2-8°C.

5.4 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets.

6 SPECIMEN

Testosterone can be determined in plasma as well as in serum of individuals who have been fasting. Do not use haemolytic, icteric or lipaemic specimens.

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

The significance of the determination of Free Testosterone can be invalidated if the sample was treated with cortisone or natural or synthetic steroids

6.1 Specimen Collection

Serum:

Collect blood by venipuncture (e.g. Sarstedt Monovette # 02.1388.001), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Individuals receiving anticoagulant therapy may require increased clotting time.

Plasma:

Whole blood should be collected into centrifuge tubes containing anti coagulant and centrifuged immediately after collection.

(E.g. for EDTA plasma Sarstedt Monovette – red cap - # 02.166.001;
for Heparin plasma Sarstedt Monovette – orange cap - # 02.165.001;
for Citrate plasma Sarstedt Monovette – green cap - # 02.167.001.)

6.2 Specimen Storage

Store specimen at -20°C if the determination is not performed on the same day of the sample collection. Freeze only once. Thawed samples should be inverted several times prior to testing.

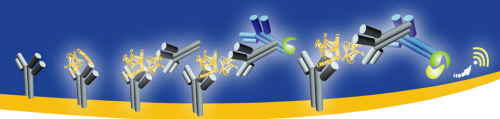
6.3 Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest calibrator, the specimens can be diluted with *Calibrator 0* and reassayed as described in Assay Procedure.

For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

- a) Dilution 1:10: 10 µL Serum + 90 µL *Calibrator 0* (mix thoroughly)
- b) Dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Calibrator 0* (mix thoroughly).



7 TEST PROCEDURE

7.1 General Remarks

- o All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- o Once the test has been started, all steps should be completed without interruption.
- o Pipetting of samples should not extend beyond ten minutes to avoid assay drift.
- o Use new disposal plastic pipette tips for each calibrator, control or sample in order to avoid cross contamination.
- o Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- o As a general rule the enzymatic reaction is linearly proportional to time and temperature.
- o Avoid the exposure of reagent TMB/H₂O₂ to directed sunlight, metals or oxidants

7.2 Assay Procedure

Each run must include a calibration curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense **20 µL** of each **Calibrator, control** and **samples** with new disposable tips into appropriate wells. Leave well A1 empty for substrate blank.
3. Dispense **200 µL Enzyme Conjugate** into each well, except for the blank well. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **60 minutes** at 37°C.
5. Briskly shake out the contents of the wells. Rinse the wells 2 times with distilled water (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
Important note:
The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6. Add **100 µL of Substrate Solution** to each well.
7. Incubate for **15 minutes** at room temperature in the dark.
8. Stop the enzymatic reaction by adding **100 µL of Stop Solution** to each well.
9. Determine the absorbance (OD) of each well at **450±10 nm** with a microtiter plate reader (against the blank). It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

7.3 Calculation of Results

1. Calculate the average absorbance values for each set of calibrators, controls and samples.
2. Construct a calibrator curve by plotting the mean absorbance obtained from each calibrator against its concentration 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 from the calibrator curve.
4. Automated method: 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this calibrator curve. Samples with concentrations higher than that of the highest calibrator have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.

8 EXPECTED VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

		Median	Mean ± 1SD pg/mL	Range pg/mL
Normal Male		14	13 ± 7	4.5 - 42
Female:	Ovulating	1.3	1.4 ± 0.9	ND - 4.1
	Oral contraceptives	0.9	1.1 ± 0.6	0.3 - 2.0
	Postmenopausal	0.8	0.9 ± 0.5	0.1 – 1.7

9 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results. Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials, results should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

