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Product Information

Free Testosterone ELISA

Catalog Number **SE120120**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description
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).

The Free Testosterone ELISA is intended for the measurement of Free Testosterone in serum or plasma. It 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 25 μL of testosterone standards, samples, 50 μL of testosterone-HRP Conjugate reagent, and 50 μL of 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, a solution 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 stop solution, and the absorbance is measured spectrophotometrically at 450 nm. A standard curve is prepared relating color intensity to the concentration of the Free Testosterone.

Components

Materials provided	96 Tests
Microwells coated with Goat anti-rabbit IgG	12 x 8 x 1
Standard: 7 vials (ready to use)	0.5 mL
Enzyme Conjugate (ready to use)	7 mL
Rabbit Anti-Testosterone Reagent (ready to use)	7 mL
TMB substrate (ready to use)	12 mL
Stop solution (ready to use)	12 mL
Wash Solution 20x Concentrated	25 mL

Reagents and Equipment Required but Not Provided.
1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbent paper or paper towel
6. Graph paper

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

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 anticoagulant 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.
5. The clinical significance of the determination of Free Testosterone can be invalidated if the patient was treated with cortisone, or natural or synthetic steroids.
6. 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

20x Wash Buffer Concentrate

Prepare 1x wash buffer by adding the contents of the bottle to 475 mL of distilled water. Store 1x Wash buffer at room temperature.

Storage/Stability

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

Procedure

Notes: 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.

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

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

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 of Testosterone Standards, controls and samples with new disposable tips into appropriate wells.
3. Dispense 50 µL of anti-testosterone reagent into each well.
4. Dispense 50 µL of 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.

Results

1. Calculate the average absorbance values for each set of standards, controls, and 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.
   Note: Samples with Free Testosterone concentration values greater than the highest standard should be diluted with standard 0 and reassayed. For the calculation of the concentrations this dilution factor has to be taken into account.

   Example of a standard Curve

	OD (450 nm)	**Concentration (pg/mL)**
Std 1	2.755	0
Std 2	2.242	0.25
Std 3	1.668	1
Std 4	1.152	2.5
Std 5	0.476	10
Std 6	0.235	25
Std 7	0.083	100

Expected Values:
It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values may be used as initial guideline ranges only:

	Age	**Range pg/mL**
Male	Adult	5–30
Female	Adult	0–3
Children	1–10	0.1–1.251

Product profile

Correlation with a Reference ELISA kit
A total of 74 sera were tested by this kit and a commercially available free-testosterone reference ELISA kit. The linear regression curve was calculated as:

$$Y = 0.82x + 0.78, r = 0.94$$

Precision
Intra-Assay

Serum	No .of Replicates	Mean pg/mL	Standard Deviation	Coefficient of Variation (%)
1	24	1.62	0.093	5.76
2	24	7.47	0.439	5.88
3	24	14.03	0.692	4.93

Inter-assay

Serum	No. of Replicates	Mean pg/mL	Standard Deviation	Coefficient of Variation (%)
1	16	1.71	0.117	6.87
2	16	7.67	0.288	3.75
3	16	14.67	0.908	6.19

Sensitivity
The sensitivity was determined by calculating the mean plus 2 SD of the standard zero point tested 20 times in the same run.

Serum	No. of Replicates	Mean pg/mL	Standard Deviation	Mean + 2 SD (Sensitivity) pg/ml
Zero standard	20	0.014	0.022	0.057

Cross-reactivity
The cross-reactions of the antibody calculated at 50% method, according to Abraham, are shown in the table:

Analyte	**% Cross reactivity**
Testosterone	100
Androstenedione	<0.1
Cortisone	<0.1
Androsterone	<0.1
DHEA-S	<0.1
Cortisol	<0.1
17α Estradiol	<0.1
Estrone	<0.1
Prednisone	<0.1
Norgestrel	<0.1
17α Ethynilestradiol	<0.1

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