GBLISA TESTOSTERONE KIT

KIT NAME	KIT SIZE	CAT. NO
GBLISA Testosterone Kit	96T	GBLTST0196T

GENUINE BIOSYSTEM

Intended Use:

Testosterone ELISA is intended to be used for the quantitative determination of Testosterone in human serum. This reagent is for In vitro Diagnostic use only.

Summary and Principle:

Testosterone is a steroid with a molecular weight of 288.4 g/mol. It is one of the most important androgens secreted into the blood. In males the testosterone is secreted primarily by the Leydig cells of the testes; in females approximately 50% of circulating testosterone is derived from peripheral conversion of androstenedione and approximately 25% from the ovary and approximately 25% from the adrenal glands. Testosterone is responsible for the development of secondary male sex characteristics and its measurements are helpful in evaluating the hypogonadal states. In women, high levels of testosterone are generally found in hirsuitism and virilization, polycystic ovaries, ovarian tumours, adrenal tumors and adrenal hyperplasia. In males high levels of Testosterone is associated with hypothalamic pituitary unit diseases, testicular tumours, congenital adrenal hyperplasia and prostate cancer.

The Testosterone ELISA assay is based on competitive reaction. Testosterone in the sample and the testosterone-HRP conjugate compete for a constant amount of Rabbit anti testosterone antibody. The microplate wells are goat coated with anti-rabbit IgG which captures the rabbit anti testosterone which, in turn, has bound with native and/or HRP-conjugated testosterone. Unbound complexes are removed by washing and then the substrate reagent is added which reacts with the HRP conjugate to form a blue colour. Stop solution is added which converts the colour to yellow that is read at 450nm. Native testosterone (sample or standard) concentration is inversely proportional to colour intensity.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Each microwell is coated with Goat Anti-Rabbit IgG. The microwells can be broken and used separately. Place unused wells or strips in the provided plastic sealable bag together with the desiccant and store at 2-8°C. Once open the wells are stable until expiry date at 2-8°C if stored as described.
Testosterone Calibrators	6x0.5ml	6x0.5ml vials containing Testosterone at concentrations of 0.0, 0.1, 0.5, 2.0, 6.0 and 18.0 ng/ml made up in a human serum matrix. THE EXACT CONCENTRATIONS ARE PROVIDED ON THE VIAL LABEL. CONCENTRATIONS GIVEN IN THE IFU ARE SUBJECT TO CHANGE. Ready to use. Once open stable until expiry date at 2-8°C.
Anti Testosterone Reagent	1x7ml	1 vial containing 7ml of Rabbit Anti-Testosterone antibody in Buffered saline. Once open, stable until expiry date at 2-8°C.
Testosterone HRP Conjugate Reagent	1x12ml	1 vial containing 12ml of HRP labelled monoclonal Anti-Testosterone antibody in Buffered saline. Once open, stable until expiry date at 2-8°C.
Wash Buffer Concentrate (50X)	1x15ml	PBS-Tween at pH 7.4. 50X concentrate. The concentrate must be diluted with 735ml of distilled water before use. Once diluted it is stable at room temperature for two months.
Substrate Solution	1x12ml	Mixture of TMB and Hydrogen Peroxide solution. Ready to use. Once open, stable until expiry date at 2-8°C.
Stop Solution	1x12ml	Diluted Sulfuric acid solution (1M) Ready to use. Once open, stable for 2 months at 2-8°C.

Plastic Sealable bag, IFU and plate covers.

Materials required but not provided:

Distilled water, Vortex mixer, Micropipettes, Incubator, Microplate Reader and Microplate washer.

Specimen Collection:

Serum should be prepared from whole blood specimen obtained by acceptable medical techniques. The kit is for use with serum samples without additives only.

Storage and Stability:

The contents of the kit will remain stable up to expiry date when stored at 2-8°C. Do not freeze. Keep all components tightly capped and without any contamination. Place unused wells in zip-lock bag provided and return to 2-8°C, under which conditions the wells will remain stable until the labelled expiry date. Seal and return all the other unused reagents to 2-8°C, under which conditions the stability will be retained until labelled expiry date.

Reagent preparation:

- Bring all reagents to room temperature (18-22°C) prior to use.
- Dilute the wash buffer concentrate with 735ml of Distilled water (yielding a total volume of 750ml). Once diluted the wash solution is stable for 2 months at room temperature. Mix well before use.

Preparation:

STEP 1

Remove the number of wells required and number each well for the assay series.

STEP 2

Addition of Samples and calibrators and Anti-Testosterone Reagent: Add 10 µl of Calibrators and Samples to each well. Then add 50 µl of Anti Testosterone Reagent. Thoroughly mix for 30 seconds.

STEP 3

Addition of HRP Conjugate: Add 100 µl of the Testosterone HRP Conjugate solution to each well.

STEP 4

Incubation: Cover the plate with the plate cover and incubate for 90 minutes at 37oC.

STEP 5 Washing: At the end of the incubation period, remove the plate cover and discard the contents of the wells. Wash each well 5 times with diluted washing buffer of 350 µl. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 6

Addition of the Substrate: Add 100 µl of Substrate Solution to each well. Mix gently for 10 seconds.

STEP 7

Incubation: Cover the plate with the plate cover and incubate for 20 minutes at room temperature. Ensure that the incubation is done in the dark.

STEP 8

Stopping the Reaction: Add 100 µl of Stop solution into each well and mix gently. Shake the plate to mix till the solution changes to yellow from blue.

STEP 9

Measurement: Read the absorbance of the wells at 450/630nm using a microplate reader within 15 minutes of adding the Stop Solution. Note down the absorbances.

Calculation of results:

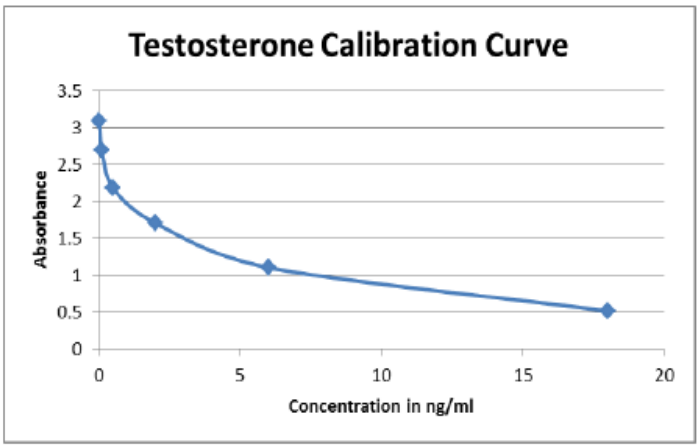
-Record the absorbances obtained from the microplate reader. Ensure that mean absorbances are calculated for duplicate measurements.

- Plot the absorbance in Y axis and Concentration in ng/ml in X axis. - Draw a point to point curve through the plotted points on a linear graph paper.

- To determine the concentration of an unknown sample, locate the absorbance of the sample on the Y axis and find the intersecting point on the curve. Read the concentration from the X axis by dropping a line from the intersecting point of the absorbance on the curve.

Example:

ID	ABSORBANCE OF CALIBRATORS	CONCENTRATION OF CALIBRATORS
CAL A	3.096	0.0 ng/ml
CAL B	2.700	0.1 ng/ml
CAL C	2.185	0.5 ng/ml
CAL D	1.709	2.0 ng/ml
CAL E	1.105	6.0 ng/ml
CAL F	0.516	18.0 ng/ml



Expected Values:

Each laboratory must establish its own normal ranges based on patient population. When randomly selected individuals were tested, the results were as follows:

Males: Prepubertal (late): 0.1 – 0.2 ng/ml
Adult: 30.0 – 10.0 ng/ml
Females: Prepubertal (late): 0.1 – 0.2 ng/ml
Follicular Phase: 0.2 – 0.8 ng/ml
Luteal Phase: 0.2 – 0.8 ng/ml
Post Menopausal: 0.08 – 0.35 ng/ml

Performance Characteristics:

1. **Intra assay Precision:**

Panel	Data no.	Mean	SD	CV%
1	20	2.26 ng/ml	0.09	3.98%
2	20	10.92 ng/ml	0.32	2.93%

2. **Inter assay Precision:**

Panel	Data no.	Mean	SD	CV%
1	20	2.14 ng/ml	0.11	5.14%
2	20	10.55 ng/ml	0.36	3.41

3. **Sensitivity:**

The minimum detectable concentration of Testosterone by this assay was found to be 0.05 ng/ml.

Cross Reactivity:

The following materials were tested for cross reactivity and the results are as follows:

Antigens	Concentration	Equivalent Testosterone	% Cross reactivity
LH	500 mIU/ml	0.0 ng/ml	0.0 %
TSH	200 uIU/ml	0.0 ng/ml	0.0 %
FSH	500 mIU/ml	0.0 ng/ml	0.0 %
HCG	1000 mIU/ml	0.0 ng/ml	0.0 %

References:

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3. Bricaire C, Raynaud A, Benotmane A et al. Selective venous catheterization in the evaluation of hyperandrogenism. J Endocrinol Invest, 1991; 14: 949-956.
4. Heinomem PK. Androgen production by pithelial ovarian tumours I postmenopausal women. Maturitas, 1991; 13: 117-122.
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