

2. Sensitivity

The sensitivity of this method is 0.002 at the 95% confidence limit.

3. Precision

- a. The Intra assay variation was determined by replicate determination (16x) of two different control sera in on assay. The within assay variability is 6.4%
- b. The Inter assay variation was determined by replicate measurements of three different control sera in 2 different lots. The between assay variability is 8%.

METHOD COMPARISON

Correlation with RIA and another commercially available Free Testosterone assay. Serum samples of 69 females and 26 males were analysed according in both test systems.

The linear regression curve was calculated as

$$Y = 0.47x + 0.378, r = 0.86$$

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.
3. Paulson JD, et al. Free Testosterone concentration in serum: elevation is the hallmark of hirsutism. Am.J.Obst. Gynecol 1977; 128:851-857.
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 PJ. 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.

2008-01-15 (Mfg: 01/08)

Warning

All of Bio-Quant ELISA Kits have not been tested for clinical use and are not approved in the United States by the FDA for diagnostic clinical use. They are components or reagents made solely for research use, further manufacturing and export use. It is the commitment of Bio-Quant customers to receive its products solely for the purpose of exportation or research, and not for the purposes of clinical diagnostic use.

BIO-QUANT DOES NOT MAKE ANY OTHER WARRANTY OR REPRESENTATION WHATSOEVER, WHETHER EXPRESS OR IMPLIED, WITH RESPECT TO THESE PRODUCTS. IN PARTICULAR Bio-Quant, INC. DOES NOT MAKE ANY WARRANTY OF SUITABILITY, NON-INFRINGEMENT, MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE OF ANY PRODUCT



BIOQUANT

FREE TESTOSTERONE ELISA

Catalog No. BQ014S (96 tests)

INTENDED USE

The free Testosterone ELISA test system is an enzyme linked immunosorbent assay (ELISA) 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. It is the principal male sex hormone and the anabolic steroid. In both males and females, it plays key roles in health and well-being. 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 free Testosterone ELISA KIT is based on the principle of competitive binding. The microtiter wells are coated with an antibody directed towards a unique antigenic site on a Testosterone molecule. An aliquot of patient sample containing endogenous Free Testosterone is incubated in the coated well with enzyme conjugate, which is an anti-Free Testosterone antiserum conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off with distilled water. The amount of bound peroxidase is proportional to the concentration of Free Testosterone in the sample. Having added the substrate solution, the intensity of colour developed is proportional to the concentration of Free Testosterone in the patient sample.

MATERIALS PROVIDED		96 TESTS
1.	Microwells coated with anti-testosterone IgG	12x8x1
2.	Standard (0-5): (6 vials ready to use)	1 ml
3.	Enzyme Conjugate (ready to use)	22 ml
4.	TMB substrate (ready to use)	14 ml
5.	Stop solution (ready to use)	14 ml

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

- Potential biohazardous materials:
The calibrator and controls 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
- This kit is designed for research use only.
- 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.
- 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.
- This test kit is designed for Research and Development use only.
- 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

- Serum: Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature.
- Plasma: Whole blood should be collected into centrifuge tubes containing anti-coagulant and centrifuged immediately after collection.
- Do not use haemolytic, icteric or lipaemic serum.
- 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
- 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
- Samples with values greater than the highest standard should be diluted with standard 0 and reassayed.

PREPARATION OF REAGENTS

STANDARDS: Before use, mix for 5 minutes with rotating mixer. For exact concentration see the labels of the standard vials.

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.

- Secure the desired number of Microtiterwells in the holder.
- Dispense 20 µl Free Testosterone Standards, controls and samples with new disposable tips into appropriate wells.
- Dispense 200 µl Enzyme Conjugate into each well.
- Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
- Incubate for 1 hour at 37°C.
- Briskly shake out the contents of the wells. Rinse the wells 2 times with distilled water. 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!

- Add 100 µl of Substrate Solution to each well.
- Incubate for 15 minutes at room temperature in the dark.
- Stop the enzymatic reaction by adding 100 µl of Stop Solution to each well.
- Read absorbance on ELISA Reader at 450 nm within 10 minutes after adding the stop solution.

CALCULATION OF RESULTS

- Calculate the average absorbance values for each set of standards, controls and patient samples
- Construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration in IU/ml with absorbance value on the vertical(Y) axis and concentration on the horizontal (X) axis
- 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.
- Automated method: Computer programs using cubic spline, 4 PL (4 Parameter Logistics) or Logit-Log can generally give a good fit.

- 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.
- Calculate the average absorbance values for each set of standards, controls and patient samples
- Construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration in IU/ml with absorbance value on the vertical(Y) axis and concentration on the horizontal (X) axis
- 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.
- Automated method: Computer programs using cubic spline, 4 PL (4 Parameter Logistics) or Logit-Log can generally give a good fit.
- 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.

QUALITY CONTROL

- Control plasma or plasma pools should be analyzed with each run of calibrators and patient samples. Results generated from the analysis of the control samples should be evaluated for acceptability using appropriate statistical methods. In assays in which one or more of the quality control sample values lie outside the acceptable limits, the results for the patient sample may not be valid.
- The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
- Control plasma or plasma pools should be analyzed with each run of calibrators and patient samples. Results generated from the analysis of the control samples should be evaluated for acceptability using appropriate statistical methods. In assays in which one or more of the quality control sample values lie outside the acceptable limits, the results for the patient sample may not be valid.
- The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.

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:

		MEDIAN	MEAN ± 1 SD pg/ml	Abs. Range pg/ml
Male		14	13 ± 7	4.5-42
Female	ovulating	1.3	1.4 ± 0.9	ND-4.1
	Taking oralcontraceptives	0.9	1.1 ± 0.6	0.3-2.0
	Postmenopause	0.8	0.9 ± 0.5	0.1-1.7

- Specificity**
The cross-reaction of the antibody calculated at 50% according to Abraham are shown in the table:

Analyte	% Cross reactivity
Testosterone	100
DHT	0.006
Androstenedione	0.005
Cortisone	0
Androsterone	0
DHEA-S	0
Cortisol	0
17α Estradiol	0
Estrone	0
Prednisone	0
Norgestrel	0
17α Ethynilestradiol	0