



Testosterone ELISA

Catalog No. BDTT37-BA (96 Tests)

REFERENCES

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INTENDED USE

The bioactiva diagnostica GmbH Testosterone ELISA Kit is intended for the quantitative measurement of Testosterone in human serum or plasma.

SUMMARY AND EXPLANATION

Testosterone (17 β -hydroxyandrost-4-ene-3-one) is a C19 steroid with an unsaturated bond between C-4 and C-5, a ketone group in C-3 and a hydroxyl group in the β position at C-17. This steroid hormone has a molecular weight of 288.4. Testosterone is the most important androgen secreted into the blood. In males, testosterone is secreted primarily by the Leydig cells of the testes; in females ca. 50% of circulating testosterone is derived from peripheral conversion of androstenedione, ca. 25% from the ovary and ca. 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 hirsutism and virilization, polycystic ovaries, ovarian tumors, adrenal tumors and adrenal hyperplasia. In men, high levels of testosterone are associated to the hypothalamic pituitary unit diseases, testicular tumors, congenital adrenal hyperplasia and prostate cancer. Low levels of testosterone can be found in patients with the following diseases: Hypopituitarism, Klinefelter's syndrome, Testicular feminization, Orchiectomy and Cryptorchidism, enzymatic defects and some autoimmune diseases. The Testosterone EIA kits are designed for the measurement of total Testosterone in human serum.

PRINCIPLE OF THE TEST

The Testosterone test kit is a solid phase competitive ELISA. The samples, working Testosterone-HRP Conjugate and anti-Testosterone-biotin solution are added to the wells coated with streptavidin. Testosterone in the patient's serum competes with the Testosterone enzyme (HRP) conjugate for binding sites. Unbound Testosterone and testosterone enzyme conjugate is washed off by washing buffer. Upon the addition of the substrate, the intensity of color is inversely proportional to the concentration of Testosterone in the samples. A standard curve is prepared relating color intensity to the concentration of the Testosterone.

MATERIALS PROVIDED		96 Tests
1.	Streptavidin Microwell coated plate	12x8x1
2.	Standard: 6 vials (ready to use)	0.5ml
3.	Enzyme Conjugate (20X): 1 bottle	0.7ml
4.	Anti-Testosterone Biotin Reagent: 1 bottle (Ready to use)	7ml
5.	Assay Diluent: 1 bottle (Ready to use)	12ml
6.	TMB Substrate: 1 bottle (Ready to use)	12ml
7.	Wash Buffer (20X): 1 bottle	25ml
8.	Stop Solution: 1 bottle (Ready to use)	12ml

MATERIALS NOT PROVIDED


1. Distilled or deionized water
2. Precision pipettes. Disposable pipette tips
3. ELISA reader capable of reading absorbance at 450nm
4. Absorbance paper or paper towel
5. Graph paper

STORAGE AND STABILITY

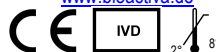
1. Store the kit at 2 - 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun, or strong light.

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For Order and Inquiries, please contact

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WARNINGS AND PRECAUTIONS

- For Laboratory Use.
- Potential biohazardous materials:
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
- Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
- 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.
- 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 HANDLING

- Collect blood specimens and separate the serum immediately.
- Typically, specimens may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20°C) for up to one month.
- Avoid multiple freeze-thaw cycles.
- Prior to assay, frozen sera should be completely thawed and mixed.
- Do not use grossly lipemic specimens.
- Please note: Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- 20X Enzyme conjugate:** Prepare 1X working solution at 1:20 with assay diluent (e.g. Add 0.1ml of the Testosterone enzyme conjugate concentrate to 1.9ml of assay diluent)
- Prepare 1X Wash buffer** by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or de-ionized water. Store at room temperature (20-25°C).
- All reagents should be brought to room temperature (20-25°C) before use.

ASSAY PROCEDURE

Before proceeding with the assay, bring all reagents to room temperature (20-25°C).

- Pipette 50µl of the standards, control or specimen into the assigned well.
- Add 100µl of working Testosterone-enzyme conjugate reagent into each well (see Reagent Preparation Section).
- Add 50µl Biotin reagent into each well. Swirl the microplate gently for 20-30 seconds to mix the reagents.
- Cover the plate and incubate for 60 minutes, at room temperature.
- Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer (see Reagent Preparation Section). Blot on absorbent paper towels.
- Add 100µl of TMB substrate reagent into each well.
- Cover the plate and incubate at room temperature, for thirty (30) minutes.
- Add 50µl of stop solution into each well, and gently mix for 15-20 seconds.
- Read the absorbance on ELISA Reader for each well at 450nm, within 15 minutes, after adding the stop solution.

CALCULATION OF RESULTS

- Calculate the mean absorbance value (A450) for each set of reference standards, controls and samples.
- Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on a linear-linear graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
- Use the mean absorbance values for each specimen to determine the corresponding concentration of Testosterone in ng/ml from the standard curve.
- Any values obtained for diluted samples must be further converted by applying the appropriate dilution factor in the calculations.

EXAMPLE OF THE STANDARD CURVE

Testosterone (ng/ml)	Absorbance (450nm)
0	2.50
0.2	2.36
0.5	1.88
2.0	0.93
6.0	0.37
18.0	0.15

LIMITATION OF THE TEST

- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

EXPECTED VALUES

It is very important to realize that normal ranges subject to many factors that play a role in determining them. Different kits may give different values and different ethnic groups may give different readings. It is advised that clients establish their own normal ranges for a group of population.

Male	
Prepubertal	0.07 – 0.2 ng/mL
Young Adults	3.0 – 9.5 ng/mL
Females	
Prepubertal	0.7 – 0.2 ng/mL
Young Adults	0.12 – 0.6 ng/mL

PERFORMANCE CHARACTERISTICS

- Precision**
Intra-Assay

Serum	No. of Replicates	Mean ng/ml	Standard Deviation	Coefficient of Variation (%)
1	24	51.57	1.5	2.91%
2	24	282.06	10.82	3.84%
3	24	386.19	14.37	3.72%

Inter-Assay

Serum	No. of Replicates	Mean ng/ml	Standard Deviation	Coefficient of Variation (%)
1	16	52.9	4.57	8.65%
2	16	275.8	14.89	5.40%
3	16	409.7	28.29	6.90%

- Sensitivity**
The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 24 times in the same run.

Serum	No. of Replicates	Mean ng/ml	Standard Deviation	Mean + 2SD (Sensitivity)
Zero standard	24	0.35	0.403	1.16 ng/ml