



Testosterone (human) ELISA Kit

(Catalog # K7417-100, 100 assays; Store at 2-8°C)

rev.10/18

I. Introduction:

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.

II. Application:

Quantitative protein detection, establishing normal range etc.

III. Specificity:

Human Testosterone

IV. Sample Type:

Serum or plasma

V. Kit Contents:

Components	K7417-100	Part No.
Microplate coated with Mouse Anti-Testosterone, 96 wells	12 stripsx8 wells	K7417-100-1
Standards (ready to use)	0.5 ml X 6	K7417-100-2.x
Assay Diluent	12 ml	K7417-100-3
Enzyme Conjugate (20X)	0.7 ml	K7417-100-4
Anti-Testosterone Biotin Reagent	7 ml	K7417-100-5
Wash Buffer (20X)	25 ml	K7417-100-6
TMB Substrate (ready to use)	12 ml	K7417-100-7
Stop Solution (ready to use)	12 ml	K7417-100-8

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips.

VII. Storage Conditions and Reagent Preparation:

Store kit at 2-8°C. Keep microwells sealed in a dry bag with desiccants. Spin tubes briefly to bring down all components to the bottom of tubes. Reagents are stable until the expiration of the kit. Do not expose reagent to heat, sun, or strong light. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

- **Wash Buffer:** Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26° C).
- **Enzyme Conjugate:** Dilute enzyme conjugate 1:20 with assay diluent in a suitable container. For example, add 100 μ l of conjugate to 1.9 ml of assay diluent. We recommend preparing diluted solution just before use.

VIII. Warning & Precautions:

- Potential biohazardous materials: The Standard contains 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, 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.
- 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.

IX. Sample Preparation and Storage:

Collect blood specimens & separate the serum immediately. Specimens may be stored refrigerated at (2-8°C) for 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 well. Don't use grossly lipemic specimens. Samples containing sodium azide should not be used in the assay.

X. Assay Protocol:

Prior to assay, bring all reagents to room temperature. Gently mix all reagents before use.

1. Pipette 50 μ l of the standards, control or specimen into the assigned well.
2. Add 100 μ l of working Testosterone-enzyme conjugate reagent into each well (see Reagent Preparation Section).
3. Add 50 μ l Biotin reagent into each well. Swirl the microplate gently for 20-30 seconds to mix the reagents.
4. Cover the plate and incubate for 60 minutes, at room temperature.



5. Remove liquid from all wells. Wash wells three times with 300 μ l of 1X wash buffer (see Reagent Preparation Section). Blot on absorbent paper towels.
6. Add 100 μ l of TMB substrate reagent into each well.
7. Cover the plate and incubate at room temperature, for thirty (30) minutes.
8. Add 50 μ l of stop solution into each well, and gently mix for 15-20 seconds.
9. Read the absorbance on ELISA Reader for each well at 450 nm, within 15 minutes, after adding the stop solution.

XI. Calculation:

1. Calculate the mean absorbance value (A450) for each set of reference standards, controls and samples.
2. 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.
3. Use the mean absorbance values for each specimen to determine the corresponding concentration of Testosterone in ng/ml from the standard curve.
4. Any values obtained for diluted samples must be further converted by applying the appropriate dilution factor in the calculations.

Example of a Standard Curve:

Standard	OD (450 nm)
Standard 1 (0 ng/ml)	2.50
Standard 2 (0.2 ng/ml)	2.36
Standard 3 (0.5 ng/ml)	1.88
Standard 4 (2.0 ng/ml)	0.93
Standard 5 (6.0 ng/ml)	0.37
Standard 6 (18.0 ng/ml)	0.15

XII. RELATED PRODUCTS:

Progesterone (mouse/rat) ELISA Kit (4715)
Progesterone (human) ELISA Kit (K7414)
Androgen Receptor Antibody (6710)

Progesterone (human) ELISA Kit For Saliva (4716)
Testosterone (mouse/rat) ELISA Kit (K7418)
Androgen Receptor Antibody (Clone 549CT16.1.4) (6711)

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