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Testosterone (mouse/rat) ELISA Kit

(Catalog # K7418-100, 100 assays; Store at 2-8^{0}C)

I. Introduction:

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. 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, or chidectomy and Cryptorchidism, enzymatic defects and some autoimmune diseases. BioVision's Testosterone EIA kit 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-Testosterone. In the incubation, goat anti-rabbit IgG-coated wells are incubated with 25 µl of Testosterone standards, controls, samples, 100 µl Testosterone-HRP conjugate reagent and 50 µl rabbit anti-testosterone reagent at room temperature for 60 min. During the incubation, a fixed amount of HRP-labeled Testosterone competes with the endogenous Testosterone in the standard, sample, or quality control serum for a fixed number of binding sites of the specific Testosterone antibody. Thus, the amount of Testosterone peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of Testosterone in the specimen increases. Unbound Testosterone peroxidase conjugate is then removed and the wells are washed. Next, a solution of TMB Reagent is then added and incubated at room temperature for 15 min., 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 450nm.

II. Application:

Quantitative protein detection, establishing normal range etc.

III. Specificity:

Mouse/rat Testosterone

IV. Sample Type:

- Serum or plasma

V. Kit Contents:

Components	K7418-100	Part No.
Microplate coated with Goat Anti-Rabbit IgG, 96 wells	12 stripsx8 wells	K7418-100-1
Standard: (0.5 ml) (ready to use)	6 vials	K7418-100-2
Rabbit Anti-Testosterone Reagent	7 ml	K7418-100-3
Assay Diluent	12 ml	K7418-100-4
Enzyme Conjugate (20X)	0.7 ml	K7418-100-5
Wash Buffer (20X)	25 ml	K7418-100-6
TMB Substrate (ready to use)	12 ml	K7418-100-7
Stop Solution (ready to use)	12 ml	K7418-100-8

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^{o}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 µl of conjugate to 1.9 ml of assay diluent. We recommend preparing diluted solution just before use.

VIII. Warning & Precautions:

- Potential biohazardous materials: The kit contains animal and/or human source components. All the human source components 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. All the animal products, if any, have been derived from animals of US origin and processed in USDA licensed facilities.
- 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.
- 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. Place the desired no. of coated strips into the holder. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipet 25 µl of Standards, control, or samples into designated wells.
3. Add 100 µl of diluted enzyme conjugate to all wells.
4. Dispense 50 µl of rabbit anti-Testosterone reagent to each well. Thoroughly mix for 30 sec.
4. Cover the plate and incubate for 60 min. at room temperature.
5. Remove liquid from all wells & wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
6. Add 100 µl of TMB substrate to all wells, mix & incubate for 15 min. at room temperature.
7. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution. It important to make sure that all the blue color changes to yellow color completely.
8. Read absorbance on ELISA Reader at 450 nm within 15 min. after adding the stop solution.

XI. Calculation: Construct the standard curve; plot the absorbance for the Testosterone standards (vertical axis) versus Testosterone standard concentrations (horizontal axis). Draw the best curve through the points. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample. 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)	2.38
Standard 2 (0.1 ng/ml)	1.75
Standard 3 (0.5 ng/ml)	1.02
Standard 4 (2.0 ng/ml)	0.59
Standard 5 (6.0 ng/ml)	0.34
Standard 6 (18.0 ng/ml)	0.17

Expected Values: Each laboratory should establish its own normal range.

XII. RELATED PRODUCTS:

Progesterone (mouse/rat) ELISA Kit (4715)
Progeststerone (human) ELISA Kit (K7414)
Androgen Receptor Antibody (6710)
Progesterone (human) ELISA Kit For Saliva (4716)
Testosterone (human) ELISA Kit (K7417)
Androgen Receptor Antibody (Clone 549CT16.1.4) (6711)

FOR RESEARCH USE ONLY! Not to be used on humans.