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CALBIOTECH
A life science company

Testosterone ELISA

Catalog No. TE187S (96 Tests)

INTENDED USE

The Calbiotech Inc Testosterone ELISA is for the quantitative determination of Testosterone concentration in human serum or plasma.

Summary AND EXPLANTION

Testosterone (17β-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, Orchidectomy 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 EIA is based on the principle of competitive binding between Testosterone in the test specimen and Testosterone-HRP conjugate for a constant amount of mouse anti-Testosterone. In the incubation, mouse anti-Testosterone coated wells are incubated with 25μl of Testosterone standards, controls, patient samples, 100 μl Testosterone-HRP conjugate reagent at room temperature for 60 minutes. 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 washed. Next, a solution of TMB Reagent is then added and incubated at room temperature for 15 minutes, 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.

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

WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. For Laboratory Use.
3. 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
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. It is recommended that serum samples be run in duplicate.
7. 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

1. Collect blood specimens and separate the serum immediately.
2. 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.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed.
5. Do not use grossly lipemic specimens.
6. Please note: Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

1. **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)
2. **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).
3. 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).

1. Pipette 25µl of the standards, control or specimen into the assigned well.
2. Add 100µl of working Testosterone-enzyme conjugate reagent to all wells (see Reagent Preparation Section).
3. 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 µl of 1X wash buffer (see Reagent Preparation Section). Blot on absorbent paper towels.
6. Add 100µl of TMB substrate reagent to all wells
7. Cover the plate and Incubate at room temperature for fifteen (15) minutes.
8. Add 50µl of stop solution to each well and gently mix for 15-20 seconds.
9. Read the absorbance on ELISA Reader for each well at 450nm within 15 minutes after adding the stop solution.

CALCULATION OF RESULTS

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 THE STANDARD CURVE

Testosterone (ng/ml)	Absorbance (450nm)
0	2.38
0.1	1.75
0.5	1.02
2.0	0.59
6.0	0.34
18.0	0.17

LIMITATION OF THE TEST

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