

CALDON BIOTECH INC.

Testosterone ELISA

Catalog No. TE080S
(96 tests)

INTENDED USE

For the quantitative determination of Testosterone concentration in human serum.

SUMMARY AND EXPLANATION

Testosterone (17 α -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 EIA 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 10 μ l of Testosterone standards, controls, patient samples, 100 μ l Testosterone-HRP conjugate reagent and 50 μ l rabbit anti-Testosterone reagent at 37C for 90 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 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of 1N HCl, and the absorbance is measured spectrophotometrically at 450nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled Testosterone in the sample. A standard curve is obtained by plotting the concentration of the standard versus the absorbance. The Testosterone concentration of the specimens and controls run concurrently with the standards can be calculated from the standard curve.

MATERIALS PROVIDED

1. Microwells coated with Goat Anti-Rabbit IgG (12x8x1 wells). 96 wells.
2. Standard: 6 vials (0.5 mL each). Ready to use.
3. Rabbit Anti-Testosterone Reagent (7 mL) Ready to use.
4. Enzyme Conjugate: 1 bottle (12 mL). Ready to use.
5. Controls: 1 & 2 (0.5 mL each) Ready to use.
6. TMB Substrate: 1 bottle (11 mL). Ready to use.
7. Stop Solution: 1 bottle (11 mL). Ready to use.

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.

WARNINGS AND PRECAUTIONS

1. 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 test kit is designed for in vitro diagnostic use only.
 - 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.
- 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.

REAGENTS PREPARATION

- All reagents should be allowed to reach room temperature (18-25° C) before use.
- Reconstitute each lyophilized standard with 2.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. Reconstituted standards should be stored sealed and are stable for 30 days at 2-8°C.

ASSAY PROCEDURE

- Secure the desired number of coated wells in the holder.

- Dispense 10 μ l of standards, specimens and controls into appropriate wells.
- Dispense 100 μ l of Testosterone-HRP Conjugate Reagent into each well.
- Dispense 50 μ l of rabbit anti-Testosterone reagent to each well. Thoroughly mix for 30 seconds. It is very important to mix completely.
- Incubate at 37°C for 90 minutes.
- Rinse and flick the microwells 5 times with distilled or deionized water. (Please do not use tap water.)
- Dispense 100 μ l of TMB Reagent into each well. Gently mix for 5 seconds.
- Incubate at room temperature (18-25°C) for 20 minutes.
- Stop the reaction by adding 100 μ l of Stop Solution to each well.
- Gently mix 30 seconds. *It is important to make sure that all the blue color changes to yellow color completely.*
- Read absorbance at 450 nm with a microtiter well reader within 15 minutes.

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.

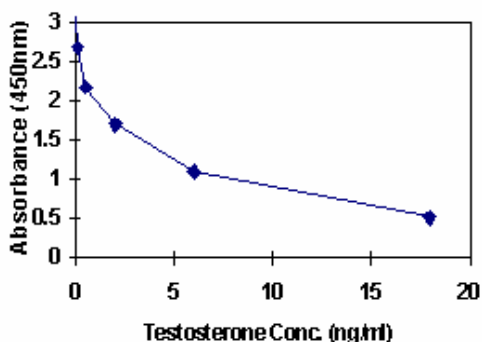
EXAMPLE OF THE STANDARD CURVE

Results of a typical standard run with optical density readings at 450nm shown in the Y axis against Testosterone concentrations shown in the X axis. **Note:** This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each laboratory must provide its own data and standard curve in each experiment.

Testosterone (ng/ml)	Absorbance (450nm)
0	3.096
0.1	2.700
0.5	2.185
2.0	1.709
6.0	1.105
18.0	0.516

Steroid Concentration

Steroid	Cross-Reactivity
Testosterone	100%
Dihydrotestosterone	0.86%
Androstenedione	0.89%
Androsterone	1.0%
17β Estradiol	0.05%
Progesterone	<0.05%
Epitestosterone	<0.05%
17-OH-Progesterone	<0.05%
Estriol	<0.05%
Cortisol	<0.05%
DHEA-Sulphate	<0.05%



EXPECTED VALUES

Each laboratory should establish its own normal range based on the patient population. The Testosterone EIA was performed on randomly selected outpatient clinical laboratory samples. The results of these determinations are as follows:

Males:

prepubertal (late)	0.1 – 0.2 ng/ml
Adult	3.0 – 10.0 ng/ml

Females:

prepubertal (late)	0.1 – 0.2 ng/ml
follicular phase	0.2 – 0.8 ng/ml
luteal phase	0.2 – 0.8 ng/ml
post menopausal	0.08 – 0.35 ng/ml

SENSITIVITY

The lowest detectable level of Testosterone in this test is 0.05 ng/ml.

SPECIFICITY

The following materials have been checked for cross reactivity. The percentage indicates cross reactivity at 50% displacement compared to Testosterone. Data on the cross-reactivity for several endogenous and pharmaceutical steroids are summarized in the following table:

$$\text{Cross-reactivity (\%)} = \frac{\text{Observed}}{\text{Testosterone Concentration}} * 100$$

LIMITATIONS OF THE TEST

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

REFERENCES:

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