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| IVD | ☣ See external label | 🌡 2°C-8°C | ∇ Σ=96 tests | REF Cat # 2095Z |

Enzyme Immunoassay for the Quantitative Determination of Testosterone Concentration in Human Serum

Testosterone

Cat # 2095Z

Testosterone Enzyme Immunoassay
For the quantitative determination of Testosterone concentration in human serum

Test	Testosterone
Method	**Enzyme Linked Immunosorbent Assay**
Principle	**Peroxidase – Conjugated Competitive ELISA**
Detection Range	**0-18 ng/ml**
Sample	**10ul serum**
Specificity	**100%**
Sensitivity	**0.05 ng/ml**
Total Time	**~ 110 min**
Shelf Life	**12-14 months**

* Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account

Introduction

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 rabbit anti-Testosterone. In the incubation, goat anti-rabbit IgG-coated wells
are incubated with 10 μl of Testosterone standards, controls, patient samples, 100 μl Testosterone-HRP conjugate reagent and 50 μl rabbit anti-Testosterone reagent at 37°C 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 450 nm. 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.

Reagents

Materials provided with the kit:

- Goat Anti-Rabbit IgG-coated microtiter wells, 96 wells
- Testosterone Reference Standards: 0, 0.1, 0.5, 2.0, 6.0 and 18.0 ng/ml. Liquids, 0.5 ml each, ready to use.
- Rabbit Anti-Testosterone Reagent (pink color), 7 ml
- Testosterone-HRP Conjugate Reagent (blue color), 12 ml
- Testosterone Control 1, Liquid, 0.5 ml, Ready to use.
- Testosterone Control 2, Liquid, 0.5 ml, Ready to use.
- TMB Reagent (One-Step) 11 ml.
- Stop Solution (1N HCl), 11 ml.

Materials required but not provided:

- Precision pipettes: 10 μl, 50 μl, 100 μl, and 1.0 ml.
- Disposable pipette tips.
- Distilled or deionized water.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Linear-linear graph paper.
- Microtiter plate reader.

Warnings and Precautions for Users

Test methods are not available which can offer complete assurance that Hepatitis B virus, Human Immunodeficiency Virus (HIV/HTLV-III/LAV), or other infectious agents are absent from the reagents in this kit. Therefore, all human blood products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation, where it exists (e.g., USA Center for Disease Control/National Institute of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories," 1984).

Specimen Collection and Preparation

1. No special pretreatment of sample is necessary.
2. Serum samples may be stored at 2-8°C for up to 24 hours, and should be frozen at −10°C or lower for longer periods. Do not use grossly hemolyzed or grossly lipemic specimens.
3. **Please note:** Samples containing sodium azide should not be used in the assay.

Storage of Test Kit and Instrumentation

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 O.D. at 450 nm wavelength is acceptable for use in absorbance measurement.

Reagent Preparation

1. All reagents should be brought to room temperature (18-25°C) before use.
2. Samples with expected testosterone concentrations over 18 ng/ml may be quantitated by dilution with diluent available from your vendor.

Assay Procedure

1. Secure the desired number of coated wells in the holder.
2. Dispense 10 μl of standards, specimens and controls into appropriate wells.
3. Dispense 100 μl of Testosterone-HRP Conjugate Reagent into each well.
4. Dispense 50 μl of rabbit anti-Testosterone reagent to each well.
5. ***Thoroughly mix for 30 seconds. It is very important to mix them completely.***
6. Incubate at **37°C** for **90** minutes.
7. Rinse and flick the microwells 5 times with distilled or deionized water. (Please do not use tap

water.)

8. Dispense 100 μl of TMB Reagent into each well. Gently mix for 10 seconds.
9. Incubate at room temperature (18-25°C) for 20 minutes.
10. Stop the reaction by adding 100μl of Stop Solution to each well.
11. Gently mix 30 seconds. *It is important to make sure that all the blue color changes to yellow color completely*.
12. Read absorbance at 450 nm with a microtiter well reader ***within 15 minutes***.
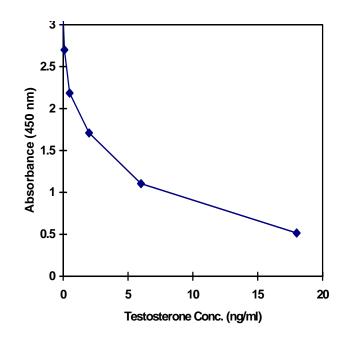
Calculation of Results

1. Calculate the mean absorbance value (A_{450}) 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 Standard Curve

Results of a typical standard run with optical density readings at
450 nm 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 (450 nm)
0	3.096
0.1	2.700
0.5	2.185
2.0	1.709
6.0	1.105
18.0	0.516

Figure: Absorbance (450 nm) vs Testosterone Conc. (ng/ml) standard curve.

Expected Normal 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

Performance Characteristics

1. Sensitivity

The minimum detectable concentration of the Testosterone ELISA assay as measured by 2 SD from the mean of a zero standard is estimated to be 0.05 ng/ml.

2. Precision

a. Intra-Assay Precision

Within-run precision was determined by replicate determinations of four different serum samples in one assay. Within-assay variability is shown below:

Samples	1	2	3	4
# Replicates	24	24	24	24
Mean Testosterone (ng/ml)	0.44	3.7	5.1	12.7
S.D.	0.03	0.4	0.4	0.6
C.V. (%)	6.4	10.0	8.3	5.0

b. Inter-Assay Precision

Between-run precision was determined by replicate measurements of six different serum samples over a series of individually calibrated assays. Between-assay variability is shown below:

Samples	1	2	3	4
# Replicates	20	20	20	20
Mean Testosterone (ng/ml)	0.45	3.4	5.0	13.3
S.D.	0.02	0.3	0.2	0.5
C.V. (%)	4.4	8.4	4.4	3.7

3. Recovery Study

Various patient serum samples of known Testosterone levels were combined and assayed in duplicate. The mean recovery was 95.3%.

PAIR NO.	EXPECTED [Testosterone] (ng/ml)	OBSERVED [Testosterone] (ng/ml)	% RECOVERY
1	8.7	9.2	105.9
2	9.3	9.6	103.6
3	6.3	5.2	83.2
4	5.0	5.0	99.9
5	2.6	3.3	127.5
6	2.4	2.3	97.5
7	0.66	0.46	70.4
8	0.61	0.46	74.6

4. Specificity

The following materials have been checked for cross reactivity. The percentage indicates cross reactivity at 50% displacement compared to Testosteronel.

Data on the cross-reactivity for several endogenous and pharmaceutical steroids are summarized in the following table:

$$\text{Cross-reactivity (\%)} = \frac{\text{Observed Estradiol Concentration}}{\text{Steroid Concentration}} \times 100$$

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%

Clinical Application

Information is cited from reference # 7

In Male:

In man, the determination of testosterone is used as an indicator for the function of the testes: low hormone levels are found in cases with Klinefelter's syndrome, cryptorchism or anorchia. Male with testosterone deficiency often present with a number of symptoms such as decreased libido, as well as decreased muscle strength, gynecomastia and infertility.

In Female:

1. **Virilizing Disorders:**
   Testosterone measurements are frequently utilized in the evaluation of virilizing disorders. **Testosterone concentrations >2.0 ng/ml may indicate androgen secreting ovarian or adrenal neoplasms.**

2. **Monitoring of Androgen Suppressing Drugs:**
   Testosterone measurements may be utilized in women for the adjustment of androgen suppressing drugs and their dosages.

3. **Pregnancy:**
   Testosterone concentrations are relatively consistent during the pregnancy.

Limitations of the Procedure
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. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

4. 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.

Quality Control

Good laboratory practice requires that controls are run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

We recommend using Bio-Rad Lyphochek Immunoassay Control Sera as controls. The Testosterone EIA kit also provides with internal controls, Level 1 and 2.

References

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Date Adopted	Reference No.
2003-01-09	DA-Testosterone-2009

Revision Date: 10/10/2009