TESTOSTERONE ENZYME IMMUNOASSAY TEST KIT
Catalog Number: 6107615

CE

ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF TESTOSTERONE CONCENTRATION IN HUMAN SERUM

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2 to 8°C.

PROPRIETARY AND COMMON NAMES

Testosterone Enzyme Immunoassay

INTENDED USE

For the quantitative determination of Testosterone concentration in human serum

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

Testosterone (antigen) in the sample competes with horseradish peroxidase testosterone (enzyme-labeled antigen) for binding onto the limited number of anti-testosterone (antibody) sites on the microplates (solid phase). After incubation the bound/free separation is performed by a simple solid-phase washing. The substrate solution (H_2O_2 + TMB) is added. After an appropriate time has elapsed for maximum color development, the enzyme reaction is stopped and the absorbance are determinated.

Testosterone concentration in the sample is calculated based on a series of standard. The color intensity is inversely proportional to the testosterone concentration of the sample.

REAGENTS

Materials provided with the kit:

- Microwell plate. 12x8 well strips. Individually separable wells. Coated with Anti-Testosterone IgG adsorbed on microplate. Packaged in an aluminum bag with a drying agent.
- Calibrators. 5 Vials x 1 mL.

  Before use, mix for 5 min. with rotating mixer.
  The standards have the following concentration of Testosterone:

	C0	C1	C2	C3	C4
ng/mL	0	0.2	1.0	4.0	16.0

- Enzyme-antigen Conjugate. Testosterone--horseradish peroxidase (HRP) conjugate. 12 mL.
- TMB Substrate solution. H_2O_2-TMB 0.25 g/L (avoid any skin contact). 12 mL
- Stop Solution. Sulphuric acid 0.15 mol/L (corrosive: avoid any skin contact). 12 mL.

Materials required but not provided:

- Multichannel pipettes and micropipettes (Precision ≥1.5%) and disposable tips.
- Microplate reader with a 450 nm filter. Reference filter of 620 or 655 nm is advisable.
- Manual or automated wash system.
- Absorbent paper of blotting the microplate wells.
- Distilled or deionised water.
- Timer.

STORAGE OF TEST KITS

The components will remain stable through the expiration date shown on the label if stored between 2-8°C in dark. Do not frezee. Do not use reagents beyond the kit expiration date.
The bag containing the microplate should be brought to room temperature before opening to avoid condensation in the wells.
Once opened the bag, microplate strips are stable for 1 month at 2-8°C in the plastic bag tightly sealed, with the silicagel.
Opened reagents are stable for 1 month at 2-8°C.

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REAGENT PREPARATION

- Stable until the expiration date of the kit when stored at 2-8ºC.
- Coated microwell strips are for one time use only.
- Calibrators, Enzyme Conjugate, Substrate Solution and Stop Solution are ready to use and need not to be diluted.
- Enzyme Conjugate: Mix gently for 5 minutes with rotating mixer. Once opened, it is stable for 6 months at + 4°C.

SPECIMEN COLLECTION AND PREPARATION

The determination of Testosterone can be performed in plasma as well as in serum of patients who have observed fast.
Store reagent at -20°C if the determination is not performed on the same day of the sample collection.
Do not use heavily hemolized samples.
The clinical significance of the determination of Testosterone can be invalidated if the patient was treated with cortisone or natural or synthetic steroids.

PRECAUTIONS

- Do not use beyond expiration date on the label.
- Do not use if reagent is not clear or if a precipitate is present.
- Do not interchange kit components.
- Follow good laboratory practices to minimize microbial and cross contamination of reagents when handling.
- Maximum precision is required for reconstitution and dispensation of the reagents.

ASSAY PROCEDURE

1. Allow all the reagents and samples to reach room temperature (20-25°C) before running the assay.
2. As it is necessary to perform the determination in duplicate, prepare two wells for each of the five points of the calibrator curve (C0-C4) and for each sample, one for Blank.
3. Pipette:

	Calibrator	Sample	Blank
Sample	---	25µL	---
Calibrators C0 –C4	25µL	---	---
Enzyme Conjugate	100µL	100µL	---
Mix well			
Incubate at 37°C for 1 hour			
Remove the contents from each well; wash the wells with 300uL of distilled water. Repeat the washing procedure by draining the water completely.			
Substrate Solution	100µL	100µL	100µL
Incubate at room temperature (20-25°C) for 15 minutes in the dark.			
Stop Solution	100µL	100µL	100µL
Read the absorbance (E) at 450 nm against Blank.			

CALCULATION OF RESULTS

CALIBRATOR CURVE

1. Mean absorbance and relative percentage
   Calculate the mean of the absorbance for each duplicate.
2. Calibration curve
   Plot the absorbance values of the calibrators against the corresponding concentrations as a lin-lin or lin-log(x-axis) diagram. Extrapolate the line passing through the points.
3. Calculation of results
   Read the concentration of samples.

EXPECTED VALUES

Each laboratory must establish its own normal ranges based on patient population.

The serum or plasma Testosterone values are comprised in the following intervals:

WOMAN: 0.2 - 1.0 ng/mL

CHILDREN: 0.1 - 0.3 ng/mL

MEN: 3.0 - 10.0 ng/mL

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. 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.

PERFORMANCE CHARACTERISTICS

A. Specificity
The cross reactions of the antibody calculated at 50% according to Abraham are shown in the table:

Testosterone	100.0	%
DHT	10.0	%
Androstenedione	0.8	%
Androsterone	0.0	%
DHEA-S	0.0	%
Cortisol	0.0	%
Cortisone	0.0	%
17 α Estradiol	0.0	%
Estrone	0.0	%
Prednisone	0.0	%

B. Precision
The inter and intra-run precision had a coefficient of variation of 3.9% and 6.2% respectively.

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C. Accuracy

The recovery of 0.2- 1.0- 4.0- 16.0 ng/mL of Testosterone added to "plasma-free" sample gave an average value (±SE) of 105.9% ± 4.2% with reference to the original concentrations.

D. Correlation with RIA

Correlation with RIA performed on the same samples:

y = -0.13 + 0.968 x
r = 0.985
n = 32
p < 0.001

This method allows the determination of Testosterone from 0.2 ng/mL to 16.0 ng/mL.

REFERENCES

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QUALITY SYSTEM CERTIFIED
ISO 9001 ISO 13485

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