

**AccuDiag™
 Testosterone
 ELISA Kit**

Cat# 2095-18



Test	Testosterone ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Competitive Immunoassay
Detection Range	0-18 ng/mL
Sample	10µL Serum
Specificity	95%
Sensitivity	0.05ng/mL
Total Time	~ 110 min
Shelf Life	12-14 Months from the manufacturing date

INTENDED USE

The Diagnostic Automation, Inc. Testosterone Enzyme Immunoassay (EIA) is intended for the quantitative determination of testosterone in human serum. Measurement of testosterone can be used as an aid in the diagnosis and treatment of various hormonal and sexual disorders.

SUMMARY AND EXPLANATION

Testosterone, (17β-Hydroxy-4-androstene-3-one), a C₁₉ steroid, is the most potent naturally secreted androgen. It is secreted by the Leydig cells of the testicles, the adrenals and the ovaries, and is the most important androgen secreted into the blood. In males, testosterone is secreted primarily by the Leydig cells of the testes; in females approximately 50% of circulating testosterone is derived from peripheral conversion of androstenedione, with the remainder from direct secretion of testosterone from the adrenal and ovarian glands.

In males, testosterone levels increase during the last trimester of fetal life due to placental and fetal pituitary gonadotropin stimulation, and then decline and increase again 30-60 days postnatally. After this, testosterone concentrations decline to low levels in childhood. At the onset of male puberty, gonadotrophin secretion leads to increased testicular production of testosterone. In adult men, serum testosterone levels show a circadian variation, with peak levels in the morning.

Testosterone is responsible for the development of secondary male sex characteristics and its measurements are helpful in evaluating hypogonadal states. In prepubertal males, elevated testosterone levels are found in both gonadotropin-dependant and independent precocious puberty (e.g. testotoxicosis, adrenal hyperplasia or adrenal tumor), as well as in androgen receptor defects. In adult males, high levels of testosterone are associated with

hypothalamic pituitary unit diseases, testicular tumors, congenital adrenal hyperplasia and prostate cancer. Likewise, in adolescent and adult males, low testosterone levels are associated with various pathologic conditions, including primary hypogonadism (e.g. testicular dysgenesis, Klinefelter syndrome) and gonadotropin deficiencies (e.g. hypopituitarism, Kallman syndrome).

In women, there is a much smaller increase in serum testosterone levels during the third trimester, followed by low levels in childhood, and a small increase during puberty. In females of all ages, elevated testosterone levels can be associated with a variety of virilizing conditions, including congenital adrenal hyperplasia, arrhenoblastoma, mixed-gonadal dysgenesis, polycystic ovarian disease, and ovarian and adrenal tumors.

Testosterone measurements may also be utilized in women for the monitoring and adjustment of androgen suppressing drugs and dosages. Testosterone concentration in serum may be raised by certain drugs, such as 19-nortestosterone, epitestosterone, ethisterone and Danazol. Similarly, common oral contraceptive drugs, drugs containing cyproterone acetate (CPA), and gonadotropin-releasing hormone (GnRH) analogues are very effective in the suppression of testosterone concentrations.

Testosterone measurement in the immediate postnatal period can aid in the differential diagnosis of ambiguous genitalia, while measurements before and after exogenous gonadotropin administration can help to detect cryptorchidism and other structural abnormalities.

The Diagnostic Automation, Inc. Testosterone EIA provides a sensitive and reliable assay for the measurement of total testosterone in human serum. The kit features a standard range of 0.1 to 18 ng/ml and will determine a minimum detectable concentration of 0.06 ng/ml. The assay provides results in 2 hours in a microtiter plate format.

TEST PRINCIPLE

The Testosterone EIA is based on the principle of competitive binding between testosterone in the test specimen and testosterone-horseradish peroxidase (HRP) conjugate, for a constant amount of rabbit anti-testosterone. In the incubation, goat anti-rabbit IgG-coated wells are incubated with testosterone standards, controls, patient samples, testosterone-HRP conjugate reagent and rabbit anti-testosterone reagent for 90 minutes. During the incubation, a fixed amount of HRP-labeled testosterone competes with the 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-HRP 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, followed by addition of TMB Reagent resulting in the development of blue color. The color development is stopped 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.

SPECIMEN COLLECTION AND PREPARATION

1. Serum should be used in the test.
2. No special pretreatment of sample is necessary.
3. Serum samples may be stored at 2-8°C for up to 24 hours, and should be frozen at -20°C or lower for longer periods. Avoid grossly hemolytic (bright red), lipemic (milky), or turbid samples.
4. **Please note:** Samples containing sodium azide should not be used in the assay.

PROCEDURAL NOTES

- Manual Pipetting: It is recommended that no more than 32 wells be used for each assay run. Pipetting of all standards, samples, and controls should be completed within 3 minutes.
- Automated Pipetting: A full plate of 96 wells may be used in each assay run. However, it is recommended that pipetting of all standards, samples, and controls be completed within 3 minutes.
- All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
- It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

MATERIALS AND COMPONENTS

Materials provided with the test kits

- Antibody-Coated Wells (1 plate, 96 wells)
Microtiter wells coated with goat anti-rabbit IgG
- Reference Standard Set (0.5 ml/vial)
Contains 0, 0.1, 0.5, 2.0, 6.0 and 18.0 ng/ml testosterone in human serum with preservatives, liquid, ready to use
- Rabbit Anti-Testosterone Reagent (7 ml)
Contains rabbit anti-testosterone in bovine serum albumin (BSA) buffer with preservatives
- Testosterone-HRP Conjugate Reagent (12 ml)
Contains testosterone conjugated to HRP
- Testosterone Control 1 and 2 (0.5 ml/vial)
Contains approximately 1.0 and 12 ng/ml testosterone, respectively, in human serum. Liquid, 0.5 ml each, ready to use
- TMB Reagent (11 ml)
Contains 3, 3', 5, 5'-TMB stabilized in buffer solution
- Stop Solution (11 ml)
Diluted hydrochloric acid (1N HCl)

Materials required but not provided

- Distilled or deionized water
- Precision pipettes: 10 µl, 50 µl, 100 µl, and 1.0 ml
- Disposable pipette tips
- Microtiter well reader capable of reading absorbance at 450 nm.
- Vortex mixer, or equivalent
- Absorbent paper
- Linear-linear graph paper

PREPARATION

- All reagents should be allowed to reach room temperature (18-25°C) before use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Samples with expected testosterone concentrations over 18 ng/ml may be quantitated by dilution with diluent available from Diagnostic Automation, Inc.

ASSAY PROCEDURE

- Secure the desired number of coated wells in the holder.
- Dispense 10 µl of standards, specimens, and controls into appropriate wells.
- Dispense 100 µl of Testosterone-HRP Conjugate Reagent into each well.
- Dispense 50 µl 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.
- Remove the incubation mixture by flicking plate contents into a waste container. Rinse and flick the microtiter wells 5 times with deionized or distilled water. DO NOT USE TAP WATER.

- Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
- Dispense 100 µL TMB Reagent into each well. Gently mix for 5 seconds.
- Incubate at room temperature for 20 minutes.
- Stop the reaction by adding 100 µL of Stop Solution to each well.
- Gently mix for 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.**

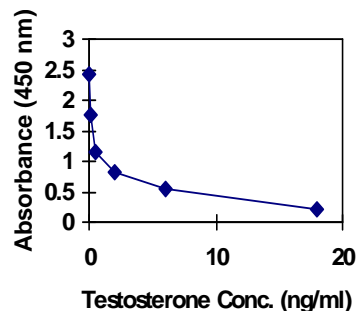
RESULTS

- Calculate the mean absorbance value (OD₄₅₀) 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 **linear-linear graph paper**, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
- Using the mean absorbance value for each sample, determine the corresponding concentration of testosterone ng/ml from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
- 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 absorbency readings at 450nm shown on the Y axis against testosterone concentrations shown on 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	2.432
0.1	1.750
0.5	1.161
2.0	0.832
6.0	0.537
18.0	0.208



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

PERFORMANCE CHARACTERISTICS

I. Accuracy

A statistical study using 109 human serum samples demonstrated good correlation with a commercially available kit as shown below.

Comparison between the Diagnostic Automation, Inc. Testosterone EIA and the DRG Testosterone kit provided the following data:

N = 102
 Correlation coefficient = 0.936
 Slope = 0.842
 Intercept = -0.122
 DAI Mean = 1.8 ng/ml
 DRG Mean = 1.4 ng/ml

II. 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.

III. 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

IV. 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

V. 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:

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

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.

QUALITY CONTROL

1. Good laboratory practice requires that quality control specimens (controls) be run with each calibration curve to verify assay performance. To ensure proper performance, control material should be assayed repeatedly to establish mean values and acceptable ranges.
2. We recommend using Bio-Rad Lyphochek Immunoassay Control Sera as controls. The Diagnostic Automation, Inc. Testosterone EIA kit also is provided with internal controls, Levels 1 and 2.
3. Controls containing sodium azide cannot be used.

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 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.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

PRECAUTIONS

1. **CAUTION:** This kit contains human material. The source material used for manufacture of this kit tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling and disposal should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.²¹
2. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
3. Do not use the reagent when it becomes cloudy or contamination is suspected.
4. Do not use the reagent if the vial is damaged.
5. Replace caps on reagents immediately. Do not switch caps.
6. Each well can be used only once.
7. Do not pipette reagents by mouth.
8. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
9. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
10. For in vitro diagnostic use.

STORAGE



1. Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. The opened and used reagents are stable until the expiration date if stored properly at 2-8°C.
3. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

INSTRUMENTATION

A microtiter well reader with a bandwidth of 10 nm or less and an optical density range (OD) of 0 to 3 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

REFERENCES

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<p>ISO 13485 ISO 9001</p>  <p> Diagnostic Automation/ Cortez Diagnostics, Inc. 23961 Craftsman Road, Suite E/F, Calabasas, California 91302 USA</p>			
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2004-03-01	AccuDiag™- Testosterone ELISA -2013		
<table border="1"> <tr> <td style="padding: 2px;">EC</td> <td style="padding: 2px;">REP</td> </tr> </table>	EC	REP	<p>CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands. www.cepartner4u.eu</p>
EC	REP		
Revision Date: 10-24-2013			