

**AccuDiag™  
Testosterone  
ELISA Kit**

REF 2095-18



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

## INTENDED USE

The Diagnostic Automation, Inc. Testosterone Enzyme-Linked Immunosorbent Assay (ELISA) 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 19-C steroid with an unsaturated bond between C<sub>4</sub> and C<sub>5</sub>, a ketone group in C<sub>3</sub> and a hydroxyl group in the β-position at C<sub>17</sub>. 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 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 DAI Testosterone ELISA kits are designed for the measurement of total testosterone in human serum.

## TEST PRINCIPLE

The DAI Testosterone ELISA 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 10µl of testosterone standards, controls, patient samples, 100µl 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-HRP 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, followed by addition of TMB Reagent and incubation 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.

## 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 or grossly lipemic specimens.
4. **Please note:** Samples containing sodium azide should not be used in the assay.

## MATERIALS AND COMPONENTS

### Materials provided with the test kits

1. Antibody-Coated Wells (1 plate, 96 wells)  
Microtiter wells coated with goat anti-rabbit IgG.
2. 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.
3. Rabbit Anti-Testosterone Reagent (7 ml)  
Contains rabbit anti-testosterone in bovine serum albumin (BSA) buffer with preservatives.
4. Testosterone-HRP Conjugate Reagent (12 ml)  
Contains testosterone conjugated to HRP.
5. Testosterone Controls 1 and 2 (0.5 ml/vial)  
Liquid, 0.5 ml each, ready to use.
6. TMB Reagent (11 ml)  
Contains 3, 3', 5, 5'-TMB stabilized in buffer solution.
7. Stop Solution (11 ml)  
Diluted hydrochloric acid (1N HCl).

### Materials required but not provided

1. Distilled or deionized water



2. Precision pipettes: 10  $\mu$ l, 50  $\mu$ l, 100  $\mu$ l, and 1.0 ml
3. Disposable pipette tips
4. Microtiter well reader capable of reading absorbance at 450 nm.
5. Vortex mixer, or equivalent
6. Absorbent paper
7. Linear-linear graph paper

## 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.<sup>21</sup>
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.

## PREPARATION

1. All reagents should be allowed to reach 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 Diagnostic Automation, Inc.
3. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.

## ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 10  $\mu$ l of standards, specimens, and controls into appropriate wells.
3. Dispense 100  $\mu$ l of Testosterone-HRP Conjugate Reagent into each well.
4. Dispense 50  $\mu$ l of rabbit anti-Testosterone reagent to each well. Thoroughly mix for 30 seconds. It is very important to mix completely.
5. Incubate at 37°C for 90 minutes.
6. 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.**
7. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
8. Dispense 100  $\mu$ L 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 100  $\mu$ L of Stop Solution to each well.
11. Gently mix for 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.

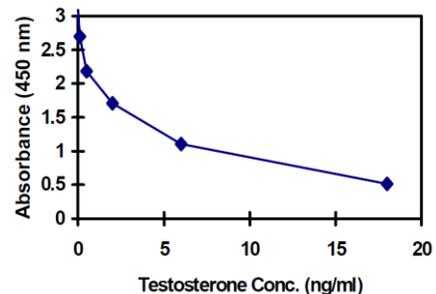
## RESULTS

1. Calculate the mean absorbance value (OD<sub>450</sub>) 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 **linear-linear graph paper**, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance values for each sample, determine the corresponding concentration of testosterone in ng/ml from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
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 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 DAI Testosterone ELISA 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. Sensitivity

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

### II. Accuracy

A statistical study using 109 human serum samples demonstrated good correlation available kit as shown below. Comparison between DAI Testosterone EIA and a commercially available Testosterone kit provided the following data:

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

### V. 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

### VI. 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

- 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.
- 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.

### 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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<b>EC</b> <b>REP</b>	<b>CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands. <a href="http://www.cepartner4u.eu">www.cepartner4u.eu</a></b>
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