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See external label



2°C-8°C



Σ=96 tests



Cat # 2924Z

Free Testosterone

Cat# 2924Z

Direct immunoenzymatic determination of Free Testosterone in serum or plasma.

Test	Free Testosterone ELISA
Method	ELISA: Enzyme Linked Immunosorbent Assay
Principle	Peroxidase – Conjugated Competitive ELISA
Detection Range	0-100pg/ml
Sample	20 ul serum
Specificity	100%
Sensitivity	0.6 pg/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.

INTENDED USE

The Diagnostic Automation Free Testosterone ELISA is Competitive immunoenzymatic colorimetric method for quantitative determination of Free Testosterone concentration in serum and plasma.

CLINICAL SIGNIFICANCE

Diagnostic Automation Free Testosterone is a steroid hormone from the androgen group. Testosterone is primarily secreted in the testes of males and the ovaries of females although small amounts are secreted by the adrenal glands. It is the principal male sex hormone and an anabolic steroid. In both males and females, it plays key roles in health and well-being.

Only 1-2% of circulating testosterone exists as unbound or free testosterone. The majority, approximately 60%, is bound to SHBG with high affinity, while the remainder is loosely bound to albumin. Both the albumin-bound and free fractions may be biologically active, while SHBG effectively inhibits testosterone action.

Testosterone effects can be classified as *virilizing* and *anabolic* effects. Anabolic effects include growth of muscle mass and strength, increased bone density and strength, and stimulation of linear growth and bone maturation. Virilizing effects include maturation of the sex organs.

Testosterone levels decline gradually with age in men.

Measurement of the free or unbound fraction of serum testosterone has been proposed as a means of estimating the physiologically bioactive hormone. Free testosterone levels are elevated in women with hyperandrogenism associated with hirsutism in the presence or absence of polycystic ovarian disease. In addition, free testosterone measurements may be more useful than total testosterone in situations where SHBG is increased or decreased (e.g. hypothyroidism and obesity).

PRINCIPLE

Free 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 enzyme substrate (H_2O_2) and the TMB-Substrate (TMB) are added. After an appropriate time has elapsed for maximum color development, the enzyme reaction is stopped and the absorbance is determined. Free Testosterone concentration in the sample is calculated based on a series of standard.

The color intensity is inversely proportional to the Free Testosterone concentration of in the sample.

Testosterone in the blood is bound to SHBG (60 %) and in lower quantity to other protein. Only the measurement of Free Testosterone (< 1% of Total Testosterone) permits the estimating of the hormone biologically active.

REAGENT, MATERIAL AND INSTRUMENTATION

1. Reagent and material supplied in the kit

1. Free Testosterone Standards 6x (1 vial = 1 mL)

STD0	REF DAS0/2924Z
STD1	REF DAS1/2924Z
STD2	REF DAS2/2924Z
STD3	REF DAS3/2924Z
STD4	REF DAS4/2924Z
STD5	REF DAS5/2924Z

2. Conjugate (1 bottle) 15 mL Testosterone-HRP conjugate. **REF** DA-C/2924Z

3. Control (1 vial = 1ml) **REF** DA-Con/2924Z

4. Coated Microplate (1 microplate breakable) **REF** DA-P/2924Z
Anti-Testosterone IgG adsorbed on microplate

5. TMB-substrate (1 bottle) 15 mL **REF** DA-T/2924Z
 H_2O_2 .TMB 0.26gr/L (avoid any skin contact)

6. Stop solution (1 bottle) 15 mL REF DA-S/2924Z
 Sulphuric acid 0.15 M (avoid any skin contact)
6. Conc. Wash Solution 10X (1 bottle = 50mL)
 NaCl 160 g/L; tween-20 10 g/L
 20 mM Phosphate buffer, pH 7.4 REF DA-W/2924Z

2. Reagents necessary not supplied

Distilled water.

3. Auxiliary materials and instrumentation

Automatic dispenser.

Microplates reader

Note

Store all reagents at 2-8° in the dark.

Open the bag of reagent 3 (Coated Microplate) only when it is at room temperature and close immediately after use.

The microplate, once opened, is stable until the expire date of kit. Do not remove the adhesive sheets on the strips inutilized

PRECAUTION

- The reagent contain Proclin 300^R as preservative.
- Avoid the exposure of reagent TMB/H₂O₂ to directed sunlight, metals or oxidants.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Do not use different lots reagents
- Do not use heavily haemolized samples.
- This method allows the determination of Free Testosterone from 0.06 pg/mL to 100.0 pg/mL.
- The clinical significance of the determination Free Testosterone can be invalidated if the patient was treated with cortisone or natural or synthetic steroids.
- If you use automatic instrument, user has responsibility to make sure that the kit has been appropriately tested.

PROCEDURE

1. Preparation of the Standard (S₀,S₁,S₂,S₃,S₄,S₅) and Control

Before use, mix for 5 min. with rotating mixer

The standard has the following concentration of FreeTestosterone:

	S ₀	S ₁	S ₂	S ₃	S ₄	S ₅
pg/ml	0	0.2	1.0	4.0	20.0	100.0

When is open is stable six months at +4°C.

2. Preparation of the Sample

The determination of Free Testosterone can be performed in plasma as well as in serum of patients who have observed fast. Store specimen at -20°C if the determination is not performed on the same day of the sample collection.

3. Preparation of the Wash Solution

Dilute the content of the vial "Conc. Wash Solution 10X" with distilled water to a final volume of 500mL prior to use. For smaller volumes respect the 1:10 dilution ratio. The diluted wash solution is stable for 30 days at 2-8°C. In concentrated wash solution is possible to observe the presence of crystals, in this case mix at room temperature until complete dissolution of crystals, for greater accuracy dilute the whole bottle of concentrated wash solution to 500mL on taking care also transfer crystals with washing of the bottle, then mix until crystal are completely dissolved.

PROCEDURE

As it is necessary to perform the determination in duplicate, prepare two wells for each of the five points of the standard curve (S_0 - S_5), two for each sample, one for Blank.

Pipette:

Reagent	Standard	Sample	Blank
Standard S_0 - S_5	20 μ L		
Control	20 μ L		
Sample		20 μ L	
Conjugate	100 μ L	100 μ L	
Incubate at 37°C for 1 hour. Remove the contents from each well, wash the wells three times with 300 mL diluted wash solution. Remove the wash solution completely.			
TMB substrate	100 μ L	100 μ L	100 μ L
Incubate at room temperature 22-28°C for 15 minutes in the dark. Pipette:			
Stop solution	100 μ L	100 μ L	100 μ L
Read the absorbance (E) at 450 nm against Blank.			

QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of Free Testosterone for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

LIMITATION OF PROCEDURE

Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipemic or haemolysed specimen(s) should similarly not be used. It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than one plate is used, it is recommended to repeat the dose response curve. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction. Plate readers measure vertically. Do not touch the bottom of the wells. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.

RESULTS

1. Mean Absorbance

Calculate the mean of the absorbance (Em) for each point of the standard curve and of each sample.

2. Standard curve

Plot the mean value of absorbance of the standards (Em) against concentration. Draw the best-fit curve through the plotted points. (es: Four Parameter Logistic).

3. Calculation of Results

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in pg/mL.

4. Range of Control: IT can be determined from the COA of the given lot. (Control's value should fall in the given range)

REFERENCE VALUE

	Media	Mean \pm 1SD pg/mL	Range pg/mL
Normal Male	14	13.0 \pm 7.0	4.5 - 42
Female:			
Ovulating	1.3	1.4 \pm 0.9	ND - 4.1
Oral contraceptives	0.9	1.1 \pm 0.6	0.3 - 2.0
Postmenopausal	0.8	0.9 \pm 0.5	0.1 – 1.7

PERFORMANCE AND CHARACTERISTICS

Precision

1. Intra Assay Variation

Within run variation was determined by replicate determination (15x) of three different serum samples in the same assay. The within assay variability is \leq 10%.

2. Inter Assay Variation

Between run variation was determined by replicate measurements of three different control sera and two serum samples in 10 different lots. The between assay variability is \leq 10%.

Sensitivity

The minimum detection limit (MDL) was calculated by linear regression from average abs standard zero and standard 1 then was dosed the 2nd s.d. of standard zero abs. The lowest detectable concentration of Free Testosterone is 0.06 pg/ml.

Specificity

The specificity was assessed by measuring the apparent response of the assay to the following potentially cross-reactive analytes and interfering substances (Anticoagulants).

The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

Analyte	% Cross reactivity
Testosterone	100
DHT	0,00008
Androstenedione	0.0043
Androsterone	0,00029
DHEA-S	0,00007
Cortisol	< 0,00001
Cortisone	< 0,00001
17 β Estradiolo	0,00005
Estrone	< 0,00001
Prednisone	< 0,00001

17 α Ethynilestradiol	< 0,00001
Norgestrel	0,00001
Danazol	<0,00001
Aldosterone	<0,00001
Sodium Citrate	<0,00001
EDTA	<0,00001
Heparin	<0,00001

Correlation

The DAI Free Testosterone ELISA was compared to present Free Testosterone RIA. Serum samples of 24 females and 17 males were analyzed according in both test systems. The linear regression curve was calculated

$$DAI = 0.957* (FT RIA) + 0.953$$

$$r^2 = 0.937$$

WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

Reference

1. McCann D, Kirkish L. J. Clin. Immunoassay 8:234-6 (1985)
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4. Odland V. et al., Clin. Endocrinology 1982;16:243-49
5. Green PJ., Clin Chem 1982;28:1237
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Date Adopted	Reference No.
2011-06-30	DA-Free Testosterone-2011



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