DIAGNOSTIC AUTOMATION, INC.
23961 Craftsman Road, Suite D/E/F, Calabasas, CA 91302
Tel: (818) 591-3030 Fax: (818) 591-8383
onestep@rapidtest.com
technicalsupport@rapidtest.com
www.rapidtest.com

IVD | See external label | 2°C-8°C | Σ=96 tests | REF Cat # 2924Z

Free Testosterone

Cat# 2924Z

Direct immunoenzymatic determination of Free Testosterone in serum or plasma.
For in vitro diagnostic use only

INTENDED USE

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 compets 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 determinated. 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) 22 mL **REF DA-C/2924Z**
Testosterone-HRP conjugate
3. Coated Microplate (1 microplate breakable) **REF DA-P/2924Z**
Anti-Testosterone IgG adsorbed on microplate
4. TMB-substrate (1 bottle) 12 mL **REF DA-T/2924Z**
H_2O_2-TMB 0.25gr/L (avoid any skin contact)
5. Stop solution (1 bottle) 12 mL **REF DA-S/2924Z**
Sulphuric acid 0.15 mol/L (avoid any skin contact)

2. Reagents necessary not supplied

Distilled water.

3. Auxiliary materials and instrumentation

Automatic dispenser.
Microplates reader

Note

Store all reagents between +2 and + 8C° 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, it stable until the expire date of kit. Do not remove the adhesive sheets on the strips inutilized

PRECAUTION

- Do not use heavily hemolized samples.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Avoid the exposure of reagent TMB/H2O2 to directed sunlight, metals or oxidants
- This method allows the determination of Free Testosterone from 0.10 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.

PROCEDURE

1. Preparation of the Standard (S_0,S_1,S_2,S_3,S_4,S_5)

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.

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.

Reagent	Standard	Sample	Blank
Standard S_0-S_5	**20 µL**		
Sample		**20 µL**	
Conjugate	**200 µL**	**200 µL**	
Incubate at 37°C for *1 hour.* Remove the contents from each well; wash the wells with 300 µL of distilled water. Repeat the washing procedure by draining the water completely			
TMB substrate	**100 µL**	**100 µL**	
Incubate at room temperature 22÷28°C for *15 minutes* in the dark.			
Stop solution	**100 µL**	**100 µL**	**100 µL**
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

1. Assay Performance

Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipemeic 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.

2. Interpretation

If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

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.

REFERENCE VALUE

	Median	Mean ± 1SD pg/mL	Range pg/mL	
Normal Male	14	13 ± 7	4.5 - 42	
Female:				
Ovulating	1.3	1.4±0.9	ND - 4.1	
Oral contraceptives		0.9	1.1± 0.6	0.3 - 2.0
Postmenopausal	0.8	0.9±0.5	0.1 – 1.7	

PERFORMANCE AND CHARACTERISTICS

Precision

1. Intra Assay Variation

Within run variation was determined by replicate determination (16x) of two different control sera in one assay. The within assay variability is 6.4%.

2. Inter Assay Variation

Between run variation was determined by replicate measurements of three different control sera in 2 different lots. The between assay variability is 8%.

3. Specificity

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

Analyte	% Cross reactivity
Testosterone	100
DHT	0.006
Androstenedione	0.0005
Androsterone	0
DHEA-S	0
Cortisol	0
Cortison	0
17α Estradiol	0
Estrone	0
Prednisone	0
17α Ethynilestradiol	0
Norgestrel	0

4. Sensitivity
The lowest detectable concentration of Free Testosterone that can be distinguished from the zero standard is 0.002 pg/ml at the 95 % confidence limit.

5. Correlation with RIA
The DAI Free Testosterone ELISA was compared to another commercially available Free Testosterone assay. Serum samples of 69 females and 26 males were analysed according in both test systems.
The linear regression curve was calculated
$y = 0.47 x + 0.378$
$r = 0.86$

6. Hook Effect
The Free Testosterone ELISA, a competitive enzyme immunoassay, shows no Hook Effect up to 400 pg/ml.

WASTE MANAGEMENT
Reagents must be disposed off in accordance with local regulations.

Reference

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Date Adopted	Reference No.
2008-01-01	DA-Free Testosterone-2008

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ISO 13485-2003

Revision Date: 5/20/08