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IVD

See external label

2°C-8°C

Σ=96 tests

REF #2924-17

FREE TESTOSTERONE

Direct immunoenzymatic determination of Free Testosterone in serum or plasma.

For in vitro diagnostic use only

Cat. No. 2924-17

1. 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 (H₂O₂) 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 are 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 albumine 1-2%, only the measurement of Free Testosterone permits the estimating of the hormone biologically active.

2. REAGENT, MATERIAL AND INSTRUMENTATION

2.1 Reagent and material supplied in the kit

- Free Testosterone Standards
S₀-S₁-S₂-S₃-S₄,-S₅ (1 bottle each) 1 mL
- 2. Conjugate (1 bottle) 6 ml
Testosterone-HRP conjugate
- 3. Coated Microplate (1 microplate)
Anti-Testosterone IgG adsorbed on microplate
- 4 TMB-Substrate (1 bottle) 12 mL
H₂O₂.TMB 0.25gr/L (avoid any skin contact)
- 5. Stop solution (1 bottle) 12 mL
Sulphuric acid 0.15 mol/L (corrosive: avoid any skin contact)

2.2 Notes

- Store all reagents between +2 and + 8°C in the dark.
- Open the bag of reagent 3 (Coated Microplate) only when it is at room temperature and close immediately after use.
- Do not remove the adhesive sheets on the strips inutilized

2.3 Reagents necessary which are not supplied with the kit

Distilled water.

2.4 Auxilliary materials and instrumentation

- Automatic dispenser.
- Microplates reader

2.5 Preparation of reagents

Standard (S₀,S₁,S₂,S₃,S₄)

Before use, mix for 5 min. with rotating mixer

The standard has the following concentration of Free Testosterone:

	S ₀	S ₁	S ₂	S ₃	S ₄	S ₅
pg/ml	0	.2	1.0	4.0	20.0	100.0
When is open is stable six months at +4°C.						

3. 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 reagent at -20°C if the determination is not performed on the same day of the sample connection.

3.1 Precaution

- Do not use heavily hemolized samples.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- 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.

4. PROCEDURE

As it is necessary to perform the determination in duplicate, prepare two wells for each of the four points of the standard curve (S₀-S₅), two for each sample, one for Blank.

Pipette:

	Standard	Sample	Blank
Sample	---	50 1	---
Standards S ₀ -S ₄	50 1	---	---
Diluted Conjugate	50 1	50 1	---

Incubate at 37°C for **1 hour**

Remove the contents from each well; wash the wells with 300 L of distilled water. Repeat the washing procedure by draining the water completely.

Pipette

	Standard	Sample	Blank
TMB-Substrate	100 1	100 1	100 1

Incubate at room temperature (20-25°C) for 15 minutes in the dark.

Pipette:

	Standard	Sample	Blank
Stop solution	100 1	100 1	100 1

Read the absorbance (E) at 450 nm against Blank.

5. STANDARD CURVE - CALCULATION OF RESULTS

5.1 Mean absorbance and relative percentage

Calculate the mean of the absorbances (Em) corresponding to the single points to the standard curve and of each sample. Express data as the percentage of the mean absorbance of S₀ (EmB₀) with the following formula:

$$(B/B_0)\% = \frac{Em}{(Em B_0)} \times 100$$

5.2 Standard curve

Plot the values of the standards expressed as (B/B₀)% on the enclosed logit-log paper.

Extrapolate the line passing through the points.

5.3 Calculation of results

Interpolate the values of the samples expressed as (B/B₀)% on the standard curve to obtain the corresponding values of the concentrations expressed in pg/mL.

6. REFERENCE VALUES

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

	N	MEDIAN	MEAN ± 1SD	ABS.RANGE
Normal Male(20-50):	35	16	15 ± 7	5.5 - 42
Female (Ovulating):	29	1.3	1.4 ± 0.9	ND - 4.1
(Oral contraceptives)	12	0.9	1.1 ± 0.6	0.3 - 2.0
(Postmenopausal)	22	0.8	0.9 ± 0.5	0.1 - 1.7

7. PERFORMANCE CHARACTERISTICS

7.1 Specificity

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

Testosterone	100.0	%
DHT	0.006	%
Androstenedione	0.0005	%
Androsterone	0.0	%
DHEA-S	0.0	%
Cortisol	0.0	%
Cortisone	0.0	%
17 α Estradiol	0.0	%
Estrone	0.0	%
Prednisone	0.0	%

7.2 Sensitivity

The sensitivity of this method, calculated as two times the S.D. from B₀, is 0.10 pg/ml when the value of (B/B₀)% is approx 90%.

7.3 Precision

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

7.4 Method comparison

Correlation with RIA and conventional equilibrium dialysis method performed on the same samples (16 male and 19 female):

Linear regression analysis of the data yielded the following statistics:

Free T. Diagnostic Automation, Inc. = 0.86 (Free T. RIA) + 0.18 pg/mL
 r = 0.97
 n = 35

7.5 Free Testosterone (% of Total Testosterone)

	N	MEDIAN	MEAN ± 1SD	ABS.RANGE
Normal Male(20-50):	35	0.45	0.5 ± 0.12	0.15 - 0.8
Female (ovulating):	29	0.5	0.55 ± 0.1	0.05 - 3.2
(oral contraceptives)	12	0.5	0.60 ± 0.1	0.01 - 1.1
(Postmenopausal)	22	0.4	0.45 ± 0.1	0.05 - 1.2

BIBLIOGRAPHY

- 1) McCann D, Kirkish L. Evaluation of Free Testosterone in serum. J. Clin. Immunoassay 1985;8:234-6
- 2) Ekins RP. Free hormones in blood J. Clin. Immunoassay 1984; 7(2): 163 - 80
- 3) Paulson JD, et al. Free Testosterone concentration in serum: elevation is the hallmark of hirsutism Am. J Obst. Gynecol 1977;128:851-7
- 4) Odland V. et al. Plasma androgenic activity in women with acne vulgaris and in healthy girls before, during and after puberty. Clin. Endocrinology 1982;16:243-49
- 5) Green PJ. Free Testosterone determination by ultrafiltration and comparison with dialysis. Clin Chem 1982;28:1237
- 6) Ekins RP. Free hormones in blood. J. Clin Immunoassay 1984; 7(2): 163-80
- 7) Wu CH. Plasma free and protein-bound testosterone in hirsutism Obstet Gynecol. 1982;60:188-94

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