AccuDiag™ Free Testosterone ELISA Kit

Cat# 2924-17

IVD See external Label 2°C 8°C Σ 96 Tests

Test	Free Testosterone ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Competitive Enzyme Immunoassay
Detection Range	0.06 / 100 pg /ml
Sample	20 µL serum/plasma
Total Time	~75 min.
Shelf Life	12 -14 Months from the manufacturing date
Specificity	100 %
Sensitivity	0.06 pg/ml

INTENDED USE

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

SUMMARY AND EXPLANATION

Diagnostic Automation Free Testosterone is a steroid hormone from the androgen group. Testosterone is primarily secreted in the test 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).

TEST PRINCIPLE

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.
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 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.

Free Testosterone concentration in the sample is calculated through a calibration curve.

Reagent Preparation

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

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	0.2	1.0	4.0	20.0	100.0

When is open is stable six months at 2-8ºC.

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 -20ºC if the determination is not performed on the same day of the sample collection. Avoid repetitive freezing and thawing of samples. The Control is ready for use.

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.

MATERIALS AND COMPONENTS

Materials provided with the test kits

Reactive Reagents

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

STD0	REF DAS0/2924-17
STD1	REF DAS1/2924-17
STD2	REF DAS2/2924-17
STD3	REF DAS3/2924-17
STD4	REF DAS4/2924-17
STD5	REF DAS5/2924-17

2. Conjugate (1 bottle) 15 mL Testosterone-HRP conjugate. REF **DA-C/2924-17**
3. Control (1 vial = 1ml) REF **DA-Con/2924-17**
4. Coated Microplate (1 microplate breakable) REF **DA-P/2924-17**
   Anti-Testosterone IgG adsorbed on microplate
5. TMB-substrate (1 bottle) 15 mL REF **DA-T/2924-17**
   H_2O_2.TMB 0.26gr/L (avoid any skin contact)
6. Stop solution (1 bottle) 15 mL REF **DA-S/2924-17**
   Sulphuric acid 0.15 M (avoid any skin contact)
7. Conc. Wash Solution 10X (1 bottle = 50mL)
   NaCI 160 g/L; tween-20 10 g/L
   0.2 M Phosphate buffer, pH 7.4 REF **DA-W/2924-17**

Materials required but not provided

1. Distilled water.
2. Automatic dispenser.
3. Microplates reader (450 nm).

Notes

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

ASSAY PROCEDURE

- **Allow all reagents to reach room temperature (22-28°C).**
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- 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 µL**		
Control	**20 µL**		
Sample		**20 µL**	
Conjugate	**100 µL**	**100 µL**	
Incubate at 37℃ 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 µL**	**100 µL**	**100 µL**
Incubate at room temperature (22÷28°C) for 15 minutes in the dark. Pipette:			
Stop solution	**100 µL**	**100 µL**	**100 µL**
Read the absorbance (E) at 450 nm against Blank within 5 min.			

RESULTS

1. **Mean Absorbance**
Calculate the mean of the absorbances (Em) for each point of the standard curve (S_0-S_5) and of each sample.

2. **Standard Curve**
Plot the values of absorbance of the standards 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

The serum concentrations of Free Testosterone are within the following ranges:

	Media	Mean ± 1SD pg/mL	Range pg/mL
Normal Male	14	13.0 ± 7.0	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

Please pay attention to the fact that the determination of a range of expected values for a "normal" population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the Manufacturer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

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.

PERFORMANCE 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 ≤10%.

2. Inter Assay Variation
Between run variations was determined by replicate measurements of three different control sera and two serum samples in 10 different lots. The between assay variability is ≤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 Estradiol	0,00005
Estrone	< 0,00001
Prednisone	< 0,00001
17α 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
$r^2 = 0.937$

LIMITATIONS OF THE PROCEDURE

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.

PRECAUTIONS

1. Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
2. All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
3. Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
4. Do not interchange kit components from different lots. The expiry dates printed on the labels of the box and of the vials must be observed. Do not use any kit component beyond their expiry date.
5. If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
6. The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
7. 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 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
8. Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
9. Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
10. Maximum precision is required for reconstitution and dispensation of the reagents.
11. Samples microbiologically contaminated, highly lipemeic or haemolysed should not be used in the assay.
12. Plate readers measure vertically. Do not touch the bottom of the wells.

WARNINGS

- This kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- Some reagents contain small amounts of Proclin 300® as preservative. Avoid the contact with skin or mucosa.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.
- This method allows the determination of Free Testosterone from 0.06 pg/mL to 100.0 pg/mL.
- The clinical significance of Free Testosterone determination can be invalidated if the patient was treated with cortisone or natural or synthetic steroids.

WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

REFERENCES

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CE

ISO 13485
ISO 9001

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EC REP	CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands. www.cepartner4u.eu
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