ELISA kits available from ADI (see details at the web site)

#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	**#1200**	Human Albumin (Urinary)
#1750	Human IgG (total)	**#1760**	Human IgM
#1800	Human IgE	**#1810**	Human Ferritin
#1210	Human Transferrin (Tf)	**#0020**	Beta-2 microglobulin
#1600	Human Growth Hormone (GH)		

#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	**#1830**	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	**#1510**	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		

#0030	Human Insulin	**#0040**	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Follicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	#**0410**	HCG-free beta

#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	**#1110**	Human Free T4 (fT4)
#1650	Human free triiodothyronine (fT3)	**#1700**	Human T3 (total)

#1850	Human Cortisol	**#1860**	Human Progesterone
#1865	Human Pregnolone	**#1875**	Human Aldosterone
#1880	Human Testosterone	**#1885**	Human free Testosterone
#1910	Human Androstenedione	**#1920**	Human Estradiol
#1925	Human Estrone	**#1940**	Dihydrotestosterone (DHT)
#1950	Human DHEA-sulphate (DHEA-S)		
#3400	Human serum Neopterin		

#3000	Human Rheumatoid Factors IgM (RF)
#3100	Human anti-dsDNA
#3200	Anti-Nuclear Antibodies (ANA)

Instruction Manual No. M-1885

Free Testosterone

ELISA KIT Cat. No. 1885, 96 Tests

For Quantitative Determination of Free Testosterone In Human Serum

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

4638 N Loop 1604 West • San Antonio• Texas 78249 • USA.
Phone (210) 561-9515 • Fax (210) 561-9544
Toll Free (800) 786-5777

Email: service@4adi.com

Web Site: www.4adi.com

ELISA KIT Cat. No. 1885 (96 tests)

For Quantitative Determination of Free Testosterone in Human Serum

Kit Components (96 tests)	Cat #
Rabbit Anti-free Testosterone Coated Strip plate, (96 wells)	1886
free Testosterone **Std. A**, 0.5 ml; 0 pg/ml	1887A
free Testosterone **Std. B**, 0.5 ml; 0.1 pg/ml	1887B
free Testosterone **Std. C**, 0.5 ml; 1.0 pg/ml	1887C
free Testosterone **Std. D**, 0.5 ml; 5.0 pg/ml	1887D
free Testosterone **Std. E**, 0.5 ml; 20 pg/ml	1887E
free Testosterone **Std. F**, 0.5 ml; 60 pg/ml	1887F
free Testosterone **Low Control** 0.5 ml	1887L
free Testosterone **High Control** 0.5 ml	1887H
Approximate concentrations are listed above**, Exact values** of **stds** and **control** (lot specific) are provided on the vials. Stability: 12 months in unopened vial or as indicated on label. Once opened, the control should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.	
Assay buffer, 15 ml	1888
Free Testosterone-**HRP Conjugate**, 300 ul (50X)	1889
Wash buffer 50 ml (10X)	W-10
TMB Substrate Soln, 16 ml	TMB-10
Stop Solution, 6 ml	T-10
Complete Instruction Manual	M-1885

Intended Use:

ADI's free testosterone kit is designed for Quantitative Determination of free Testosterone in Human Serum. For In Vitro Research use Only (RUO)

Introduction

Testosterone is a C-19 steroid secreted from the testis and the adrenal cortex in men and from the adrenal cortex and ovaries in women. Testosterone is also produced by peripheral tissues from androstenedione, which is of little physiological significance in men, however in women about half of the circulating testosterone is derived from this origin. Testosterone measurements are used mainly for clinical evaluation of hypogonadism in males and hyperandrogenic states in females.

Testosterone circulates in the blood bound to three proteins: sex hormone binding globulin (60-80%), albumin and cortisol binding globulin. Only about 1-2% of the total circulating testosterone remains unbound or free. Even though it is still under investigation, most researchers accept the free testosterone determination as a measure of the biologically active fraction. Free testosterone determinations are recommended to overcome the influences caused by variations in transport proteins on the total testosterone concentration.

INTRA-ASSAY PRECISION

Three samples (1.17, 15.96, 62.46 pg/ml) were assayed ten times each on the same calibrator curve. The values were SD 0.20, 0.79, 2.95 pg/ml and CV% 11, 4.9, 4.7% respectively.

INTER-ASSAY PRECISION

Three samples (0.97, 25.81, 75.81 pg/ml) were assayed ten times each on the same calibrator curve. The values were SD 0.12, 1.36, 6.66 pg/ml and CV% 12.4, 5.3, 8.8 respectively.

EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values. The following reference range (pg/ml) were established:

Males (N=71; median 12.3; central 95% range 4.25-30.37; Absolute range=3.8-34.1 pg/ml)
Males (N=60; median 1.03; central 95% range 0.04-4.18; Absolute range=0.01-7.01 pg/ml)

EFFECT OF SEX HORMONE BINDING GLOBULIN (SHBG)

The purpose of this study was to investigate a possible interference caused by the binding of SHBG to the free testosterone-horse radish peroxidase conjugate. A charcoal-stripped human serum pool was spiked precisely with SHBG at concentrations ranging from 6-200 µg/ml and was assayed with the Direct Free Testosterone ELISA Kit. Results tabulated below (in pg/ml):

SHBG Added	OD 450nm	Percent B/B_o (%)
0 µg/ml	2.34	100.0
6.25 µg/ml	2.33	99.7
12.5 µg/ml	2.27	97.2
50 µg/ml	2.14	91.6
200 µg/ml	2.10	89.7

The results showed bound values between 90-100% of B/Bo (Bo=unspiked serum) even at higher than normal (0.5-5 µg/ml) SHBG levels. In conclusion, the results showed that there was no significant influence by SHBG in the Direct Free Testosterone Direct ELISA kit.

EFFECT OF HUMAN SERUM ALBUMIN (HSA)

The purpose of this study was to investigate a possible interference of human serum albumin (HSA) on the assay procedure. HSA was added to three patient samples at concentrations of 1.25, 2.5 and 5.0 g/dl. All samples were assayed with the Free Testosterone ELISA Kit and yielded the following results (in pg/ml):

Sample	Added HSA g/dl			
	0	1.25	2.5	5.0
1	0.52	0.34	0.54	0.53
2	15.8	14.2	12.5	10.9
3	26.2	23.0	21.0	18.6

The results demonstrate no significant influence of added HSA on the three patient serum samples.

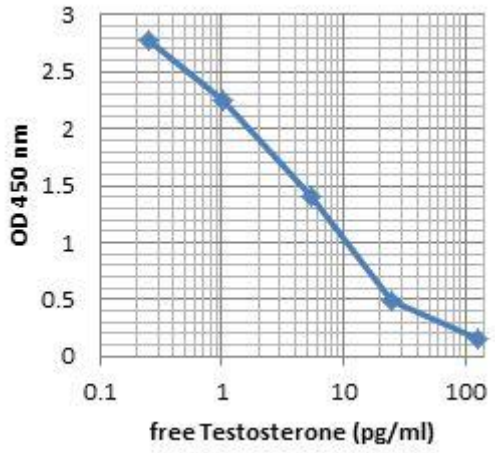
Species reactivity

This kit has been designed and tested for human serum samples. It may be optimized for other biological fluids. It has not been tested in animals (rat, mouse, etc). It will depend upon the crossreactivity of the human antibodies used in the kit with a given animal's hormones/proteins. Since the steroid hormone is the same in all species, this kit should work in most species as long as the sample cocn is within the range of this kit.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean $A_{450\ nm}$	Calculated Concn (pg/ml)
A1, A2	**Std. A** (0 pg/ml)	2.779	
B1, B2	**Std. B** (0.1 pg/ml)	2.586	
C1, C2	**Std. C** (1.0 pg/ml)	0.915	
D1, D2	**Std. D** (5 pg/ml)	0.467	
E1, E2	**Std. E** (20 pg/ml)	0.266	
F1, F2	**Std. F** (100 pg/ml)	0.150	

NOTE: These data are **for demonstration purpose only**. A complete standard curve must be run in every assay to determine sample values.



PERFORMANCE CHARACTERISTICS

SENSITIVITY: The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator A (based on 10 replicate analyses) minus 2 SD. Therefore, the sensitivity of the free Testosterone ELISA kit is **0.018 pg/mL.**

SPECIFICITY (CROSS REACTIVITY)

The following compounds were tested for cross-reactivity with the free Testosterone ELISA kit with testosterone cross-reacting at 100%.

Testosterone (100%), 5α-DHT (5.2%), Androstenedione (1.4%), Androstanediol (0.8%), Progesterone (0.5%), Androsterone (0.1%). The following steroids were tested but cross-reacted at less than 0.1%: Aldosterone, Andrenosterone, Cholesterol, Corticosterone, Dehydroepiandrosterone, Dehydroepiandrosterone Sulfate, Epiandrosterone, 17β-Estradiol, Estriol and Pregnenolone

PRINCIPLE OF THE TEST

Free Testosterone ELISA kit is based on competitive binding of human free Testosterone from serum samples and enzyme-labeled Testosterone to free-Testosterone specific antibodies immobilized on microtiter well plates. After a washing step, chromogenic substrate is added and color developed. The enzymatic reaction (blue color) is inversely proportional to the amount of free Testosterone present in the sample. The reaction is terminated by adding stopping solution (converts blue to yellow). Absorbance is then measured on a microtiter well ELISA reader at 450 nm. and the concentration of free Testosterone in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (20-100 μl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate shaker (orbital shaker), plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The Alpha Diagnostic International Free Testosterone ELISA test is intended for *in vitro research* use only. The reagents contain thimerosal or Kathon as preservative; necessary care should be taken when disposing solutions. The Control Serum has been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum.. If sera cannot be immediately assayed , these could be stored at -20^{o}C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

REAGENTS PREPARATION

Dilute Wash buffer (10x) in distilled water.

Prepare 1X solution of free Testosterone-HRP conjugate. Dilute 20 ul stock conjugate per ml of assay buffer. (200 ul in 10 ml for complete 96-well plate).

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8^{o}C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. The unused portions of the standards should be stored at 2-8^{o}C or stored frozen in small aliquots.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

1. Label or mark the microtiter well strips to be used on the plate. Dilute the enzyme conjugate (1:50) with assay buffer and wash buffer (1:10) with water.

2. Pipet **25 ul of standards**, control, and serum samples into appropriate wells in *duplicate*.

3. Add **100 ul of diluted enzyme conjugate** into each well**.** Mix gently. Cover the plate and incubate for **60 minutes** at 37oC with gentle shaking. Failure to shake will decrease the total absorbance values.

4. Aspirate and **wash the wells 3 times** with 300 ul of running tap water or distilled water. We recommend using an automated ELISA plate Washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

5. Add **150 ul TMB substrate**. Mix gently. Cover the plate and incubate for **15 minutes** at 37oC.

6. Stop the reaction by adding **50 ul of stop solution** to all wells at the same timed intervals as in step 6. Mix gently.

7. Measure the **absorbance at 450 nm** using an ELISA reader within 30 min.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4^{o}C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

Limitations

1. All the reagents within the kit are calibrated for the direct determination of free testosterone in human serum. The kit is not calibrated for the determination of free testosterone in other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Samples reading higher than 100 pg/ml should not be diluted. Dilution will alter the equilibrium between free testosterone and serum proteins.
5. The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.

CALCULATION OF RESULTS

1. Calculate the mean optical density of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter curve is recommended.
3. Calculate the mean optical density of each unknown duplicate.
4. Read the values of the unknowns directly off the calibrator curve.

COMPARATIVE STUDIES

The ADI Direct Free Testosterone ELISA Kit (y) was compared with a competitors Free Testosterone Coated Tube RIA Kit (x). The comparison of 61 serum samples yielded the following linear regression results:
y (ADI)=1.0137x (competitor) +0.6404; r=0.89