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 Folicle 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 (toal)

#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-1880

TESTOSTERONE

ELISA KIT Cat. No. 1880

For Quantitative Determination of Testosterone In Human Serum

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

TESTOSTERONE ELISA KIT Cat. No. 1880

Kit Contents: (reagents for 96 tests)

Components	Cat. #
Anti-Rabbit IgG Coated Microwell strip **plate** (96 wells)	1881
Testosterone **Std. A** (0 ng/ml), 10 ml	1882 A
Testosterone **Std. B** (0.08 ng/ml), 0.5 ml	1882 B
Testosterone **Std. C** (0.42 ng/ml), 0.5 ml	1882 C
Testosterone **Std. D** (1.67 ng/ml), 0.5 ml	1882 D
Testosterone **Std. E** (5 ng/ml), 0.5 ml	1882 E
Testosterone **Std. F** (16.7 ng/ml), 0.5 ml	1882 F
Exact stds values may vary from lot to lot and exact values given on the vial that should be used to plot the std. curve.	
Testosterone **Low & High Controls** (exact values printed on vial), #1880LC-HC	
Testosterone **Assay Buffer,** 15 ml	1883
HRP **Conjugate(50X)**, 0.3 ml; Dilute 1:50 with assay buffer	1884
Wash Buffer Conc. (10X), 50 ml *(dilute with water)*	W - 10
HRP substrate **Solution** ; 16 ml	TMB - 1880
Stop solution, 6 ml	ST - 1880
Complete Instruction Manual	M1880

Introduction

Testosterone is one of the most important male sex hormone. It is responsible for genital development, beard growth, muscle development, and general male characteristics. The measurement of serum or plasma levels is an index of Leydig cell functions and high or low values correlate well with hypo or hyper gonadism. In females, the adrenals and ovaries produce small amounts of testosterone. High levels of testosterone in females indicates excessive androgen production and found in progressive hirsutism an virilization, Cushing's syndrome, and a deficiency in one or more of the specific enzymes required for normal steroid biosynthesis. Testosterone in man and boy is related to the investigation of testicular dysfunction and is used to monitor the treatment of patients with congenital adrenal hyperplasia. In newborns or young patients, testosterone levels are used in ambiguous genitalia and isolated micropenis.

ADI's Testosterone ELISA kit provides for the measurement of Testosterone in human serum.

3. ACCURACY/RECOVERY

Two serum samples were spiked with known conc. of testosterone. The Testosterone values were measured and % of recovery was determined.

Initial Values (ng/ml)	Observed3 values (ng/ml)	Expected values (ng/ml)	Recovery (%)
Sample A Unspiked	0.14		
+ 2.5 ng/ml	2.10	2.64	80
+ 5.0 ng/ml	4.40	5.14	86
+ 10 ng/ml	11.50	10.14	113
Sample B Unspiked	6.8		
+ 2.5 ng/ml	9.25	9.3	99
+ 5.0 ng/ml	12.0	11.80	102
+ 10 ng/ml	18.0	16.80	107

3. LINEARITY

Two serum samples were diluted with Std. A. The Testosterone values were measured and % of recovery was determined.

Initial Values (ng/ml)	Observed values (ng/ml)	Expected values (ng/ml)	Recovery (%)
Sample A Undiluted	5.0		
Dilution 1:2	2.8	2.5	112
Dilution 1:4	1.0	1.25	80
Dilution 1:8	0.66	0.625	106
Sample B Undiluted	8.0		
Dilution 1:2	3.9	4.0	98
Dilution 1:4	2.0	2.0	100
Dilution 1:8	1.15	1.0	115

4. Species crossreactivity

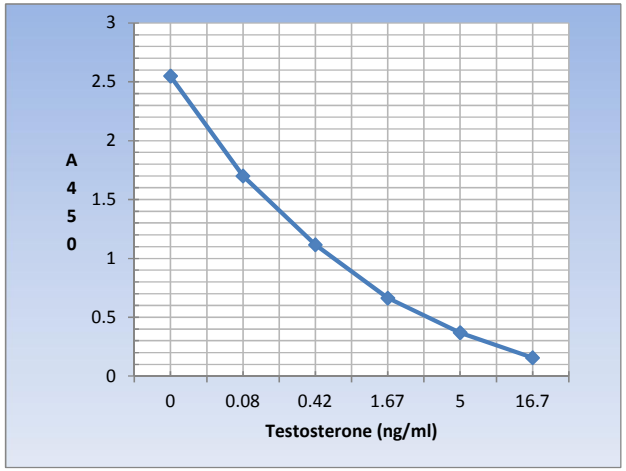
Testosterone kit has been designed and tested for human serum samples. It may be optimized for other biological fluids. Since the steroid hormone is the same in all species, this kit should work in most species as long as the sample concentrations are within the range of this kit. ADI's Human testosterone ELISA kit has been used in mouse samples.

Bhat GK, 2005 J. Androl. 27: 302 – 310, mouse testosterone
Bhat GK, 2003 Biol. Reprod. 69, 30-36 mouse testosterone
Gaddipati J (2004) J. Exp. Ther. Oncol. 4, 203-212

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples (ng/ml)	Net Mean $A_{450\ nm}$	Calc. Conc ng/ml
A1, A2	**Std. A** (0 ng/ml)	2.549	
B1, B2	**Std. B** (0.08 ng/ml)	1.699	
C1, C2	**Std. C** (0.42 ng/ml)	1.115	
D1, D2	**Std. D** (1.67 ng/ml)	0.662	
E1, E2	**Std. E** (5 ng/ml)	0.369	
E1, E2	**Std. F** (16.7 ng/ml)	0.155	
Sample 1		1.252	

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical std. assay curve (do not use this for calculating sample values)

2. PRECISION

Intra-assay precision:

	Sample A	Sample B
N	10	10
Mean (ng/ml)	0.185	7.475
S.D.(ng/ml)	0.0216	0.827
C.V. (%)	11.7	11.1

Inter-assay precision:

	Sample A	Sample B
N	10	10
Mean (ng/ml)	0.166	1.43
S.D.(ng/ml)	0.018	0.15
C.V. (%)	11.10	10.40

PRINCIPLE OF THE TEST

Testosterone ELISA kit is based upon competitive solid phase ELISA. The patient sample competes with enzyme-linked Testosterone for a fixed and limited number of antibody binding sites. In the assay, the Testosterone standard or samples sera are incubated with Testosterone-HRP conjugate and anti-Testosterone. In this solid-phase system, the antibody bound Testosterone will remain on the well while unbound Testosterone will be removed by washing. A color (blue) is developed when the substrate, TMB is mixed with the antibody bound Testosterone-HRP conjugate. After a short incubation, the enzyme reaction is stopped (blue color turns yellow) and the intensity of the color (yellow) is measured using an ELISA plate reader. The color is inversely proportional to the concentration of Testosterone in the sample.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (10-100 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader, plate shaker.

PRECAUTIONS

The Alpha Diagnostic International Testosterone ELISA test is intended for *in vitro research* use only. The reagents contain thimerosal 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.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), Diluted HCl (stop solution), and Proclin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

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 testosterone is to be measured in plasma then heparin should be used. Centrifuge for 10 min and carefully remove the plasma layer and store at 4oC. 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.

Azide and thimerosal at concn. >0.01% interfere in this test. Therefore, samples containing high concn. Of preservative may give high values.

Reagent Preparation

1. **Wash buffer (10x).** Dilute with water (50 ml stock in 450 ml water). Store at 4oC.
2. **Enzyme conjugate (50 X)**. Dilute with assay buffer. Prepare 1 ml per strip of 8 wells or 10 ml for the entire plate (200 ul enzyme stock into 9.8 ml of assay buffer). Prepare as needed. Do not store diluted solutions.

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. HRP substrate should be colorless at the time of use. If solutions have turned light blue in color, these should be replaced. Do not expose these solutions to strong light during storage or use. The unused portions of the standards should be frozen in suitable aliquots for long-term use.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).* **Dilute wash buffer (1:10) with distilled water (10 ml stock in total of 500 ml). Dilute enzyme conjugate conc.(50X) 1:50 with assay buffer.**

1. Pipet **50 ul stds. and samples** in duplicate into appropriate wells.

2. Pipet **100 ul of enzyme conjugate** into each well. Mix gently. Cover the plate and **incubate on a plate shaker** (approx. 200 rpm) at room temp. for **60 min.**

3. Remove reaction mixture and wash 3X with wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

4. Dispense **150 ul TMB substrate per well**. Mix gently for 5-10 seconds. Cover the plate and incubate on plate shaker (approx. 200 rpm) at room temp. for **15 minutes.** Blue color develops in standards and positive wells. Note it is possible to vary the time $\pm$ 5 min to get A450 in std zero to about 2.0-2.5.

5. Stop the reaction by adding **50 ul of stop** solution to all wells**.** Mix gently. 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. 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.

CALCULATION OF RESULTS

1. Plot the concentration (X) of each reference standard against its A450 using a semi-log paper. Draw a point-to-point line through the mean of the duplicate point. If ELISA reader software then use 4-parameter curve. Do not force a straight line.
2. Obtain the value of sample Testosterone by standard curve. The data given in the example is for demonstration purpose only and must not be used in place of data for each assay.

DILUTION OF SAMPLES and LIMITATIONS

It is recommended that each laboratory must determine its own normal and abnormal ranges. Extrapolation of Testosterone values beyond the standard curve may yield variable results. Samples containing >20 ng/ml Testosterone can be diluted with 0 standard (no more than 1:8 dilution) and retested. The results must be multiplied by dilution factor. Controls from other manufacturers may contain **serum preservatives (azide or merthiolate)** incompatible with ADI's ELISA reagents should not be used. Whenever laboratory data conflict with clinical findings or impressions, clinical judgment should be exercised and additional evaluation undertaken. Grossly hemolyzed or lipemic samples may give erroneous results.

Danazol metabolite may competitively displace testosterone from plasma protein; therefore, the values for testosterone in Danazol-treated patients should be appropriately corrected before interpretation

INTERPRETATION EXPECTED VALUE

1. It is recommended that each laboratory should determine its own normal and abnormal range. The following values can be used as preliminary guidelines until the laboratory establishes its own normal values: **Men** (3.0-20.0 ng/ml), **Women** (0.1-2.5 ng/ml).

PERFORMANCE CHARACTERISTICS

Specificity

The rabbit polyclonal antibody used in this kit is very sensitive and specific for Testosterone. The following compounds were tested for crossreactivity of the assay: Testosterone (100%), 5-a-dihydrostestosterone (5.0%), Androstenedione, 11-oxystestosterone, Epiandrosterone, 5-beta-DHT, 5-a-androstan-3-a, 17b-estradiol, 17b-Diol, 5-a-androstan-3, 17 dione, Androsterone, Cortisol, Dehydroepiandrosterone, Estriol, Estrone, Progesterone, Corticosterone, Danazol, 11-b-hydroxytestosterone (0%)..

Sensitivity

The minimal detectable conc. of Testosterone is estimated to be **0.022 ng/ml**. The minimal detectable conc. is defines as the concn. of Testosterone, which corresponds to the absorbance, that is 2 S.D. smaller than the mean abs. Value of the zero std.