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		

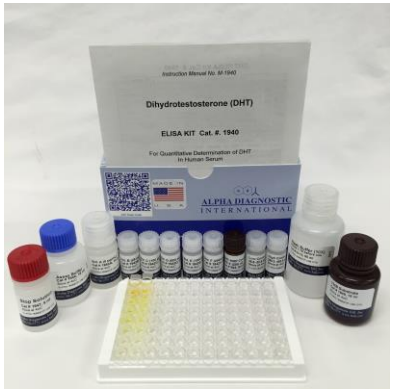
#3000	Human Rheumatoid Factors IgM (RF)
#3100	Human anti-dsDNA
#3200	Anti-Nuclear Antibodies (ANA)

Instruction Manual No. M-1940

Dihydrotestosterone (DHT)

ELISA KIT Cat. # 1940, 96 Tests

For Quantitative Determination of DHT
In Human Serum



For In Vitro Research Use Only

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DHT ELISA Kit Cat. #. 1940

Kit Components	96 tests
Anti-DHT coated strip plate (96 wells), Cat. 1941	1 plate
DHT **Std. A** (0 pg/ml), 10 ml , Cat # 1942A	1 vial
DHT **Std. B** (25 pg/ml), 0.6 ml , Cat # 1942B	1 vial
DHT **Std. C** (100 pg/ml), 0.6 ml , Cat # 1942C	1 vial
DHT **Std. D** (500 pg/ml), 0.6 ml , Cat # 1942D	1 vial
DHT **Std. E** (1000 pg/ml), 0.6 ml , Cat # 1942E	1 vial
DHT **Std. F** (2500 pg/ml), 0.6 ml , Cat # 1942F	1 vial
DHT Control Serum **Low & High**, 0.6 ml; (Lot specific values printed on vials)	2 vial
Assay buffer, 15 ml; Cat # 1 9 4 4	1 bottle
DHT-HRP Conjugate (100X), 200 ul Cat # 1 9 4 5 , **Dilute 1:100 in assay buffer before use**,	1 bottle
TMB substrate, 16 ml, Cat # 1946	1 bottle
Stop solution (1N H2SO4), 6 ml; Cat. # 1947	1 bottle
Wash buffer (10X), 50 ml, **Dilute 1:10** in distilled water	1 bottle
Instruction Manual, M - 1 9 4 0	1

Intended Use: ADI's DHT ELISA is direct competitive ELISA kit for the measurement of DHT in serum. It is not validated for other biological fluids. For In Vitro Research Use Only (RUO).

Introduction

5-α-Dihydrotestosterone (DHT) is a C19 androgenic steroid hormone similar to testosterone and androstenedione. The bulk of the androgen production takes place mainly in the Leydig cells of testes. Androgens circulate in the blood bound to proteins, especially Sex hormone binding globulin (SHBG) and albumin. A trace amount of these steroids in the unbound form in the blood and are referred to as the free fractions. DHT has at least times the binding affinity for SHBG than testosterone. In males about 70% of DHT is derived from peripheral conversion of testosterone. However, in females most of the DHT is derived from the androstenedione.

The major organ to neutralize androgens is the liver. Therefore, in the liver the steroid hormones are chemically modified that inactivate the hormone. Some metabolite are formed and returned to the circulation before renal excretion. Therefore, elimination of steroids from the body is done through the urine.

Clinical Importance

- In Klinefelter's syndrome the DHT level is much lower than that found in normal men.
- IN idiopathic hirsutism about 40% of the patients have an increased level of DHT.
- In polycystic ovaries (PCO) about 35% of the patients have an increased DHT levels.
- The DHT levels in young people are much higher than those found in normal older people, hence androgen production increases at puberty which gives rise to masculanizing characteristics. It has been demonstrated that the human testes produces DHT, which originate in the somniferous tubules. Therefore in tubular damage the production of DHT is impaired, which causes a decrease in the levels of plasma DHT (patients with germinal cell aplasia and azoospermia).

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

Based on sixteen replicate determinations of the zero standards, the minimum DHT concentration detectable using this assay is 6 pg/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

2. PRECISION

Intra-assay precision: Two serum samples (mean DHT concentrations 348.62 and 913.39 pg/ml) were run in 10 replicates. The samples showed good intra-assay precision with %CV of 10 and 11 with SD± 38.00 and 91.93, respectively.

Inter-assay precision: Two serum samples were run in duplicate in sixteen independent assays. The samples showed good inter-assay precision (3-7 % CV). The actual values were: mean 343.09 and 32.60 pg/ml.

3. LINEARITY

Three samples (with original DHT concentration of. 495.8, 569.6, 641.0 pg/ml) were diluted (1:2, 1:4, and 1:8) with the assay buffer and their final DHT values determined. The samples showed excellent mean recoveries of about 101% (range 97-107%).

4. Recovery

Three samples (with original DHT concentration of. 766, 418, 439 pg/ml) were spiked with calibrators D, E, and F and their final DHT values determined. The samples showed excellent mean recoveries of about 96% (range 92-103%).

5. SPECIFICITY AND CROSSREACTIVITY

The specificity of DHT ELISA kit was determined by measuring interference from high concentrations of the following:

DHT	100%	Testosterone	8.7%
5-beta-DHT	2%	Androstenedione	0.2%

Dehydroepiandrosterone sulfate, 17-beta-estradiol, Estriol, Estrone, Progesterone, 17-OH-Progesterone, Cortical pregnenolone <0.01%

6. Correlative Study

The ADI's DHT ELISA kits was compared with coated tube RIA kit by analyzing 10 patient samples values from men and women. The regression analyses showed good correlation (0.933) between these two methods.

7. **Species crossreactivity**- ADI's human DHT ELISA kit has been used to measure DHT in mice sera (refs 3-4).

General References: (1) Bassett, R.M (1980) Medical Lab. Science, 37:31; Baxendale, P.M., et al, (1983) Clinical Endocrinology, 18:447; Brooks, R.V., Androgens. Physiology and pathology In: Makin, H.L.J. ed., Biochemistry of steroid Hormones, 2nd ed., Oxford Blackwell Scientific Publications, 565:1984.

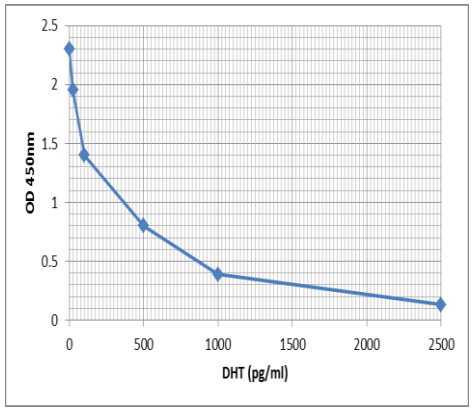
Citations of ADI's DHT ELISA Kit

(2) Pechersky AV (2002) Int. J. Andrology. 25:119-125 (Human serum)
(3) Dienstknecht T (2004) Cytokine, 25, 110-118 (mouse serum)
(4) Zhang L (2003) Cancer Res. 63: 4552 – 4560 (mouse serum)

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean $A_{450\ nm}$	Calculated Concn (ng/ml)
A1, A2	**Std. A** (0 pg/ml)	2.300	
B1, B2	**Std. B** (25 pg/ml)	1.952	
C1, C2	**Std. C** (100 pg/ml)	1.068	
D1, D2	**Std. D** (500 pg/ml	0.357	
E1, E2	**Std. E** (1000 pg/ml)	0.214	
F1, F2	**Std. F** (2500 pg/ml)	0.130	
G1, G2	**Sample 1**	0.511	300 pg/ml

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.



3_ADI_ELISA

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

PRINCIPLE OF THE TEST

DHT ELISA kit is based on simultaneous binding of DHT from samples and DHT-HRP conjugate to anti-DHT immobilized on the microtiter well plates. Therefore, free DHT and enzyme-bound DHT compete for limited antibody bound to the pale. In the absence of free DHT, there is a maximum amount of DHT-enzyme bound to the plate. At the end it will produce the maximum color. As the amount of free DHT (samples or std) increases, it reduces the amount of enzyme bound to the plate. The color (blue) produced is inversely proportional to the concentration of DHT in the sample. A std. curve is constructed by generating color from no DHT (std. A, 0 pg/ml DHT) to the highest std (Std E, 2500 pg/ml DHT). Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm. and the concentration of DHT in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-1000 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate shaker (orbital shaker), plate washer (recommended) and ELISA plates Reader.

PRECAUTIONS

The Alpha Diagnostic International DHT ELISA kit 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 stds./controls sera may contain human serum that has been 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 can not 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 FOR THE ASSAY AND STORAGE

HRP Conjugate. Dilute 1:100 in assay buffer (prepare 11 ml for a full 96 well plate. 110 ul in 11 ml of the assay buffer). Do not store diluted solution. Prepare in required amounts only. Store stock solution at 4oC.

Wash Buffer Concentrate

Dilute 1:10 in distilled water (50 ml stock in 450 ml) before use. Occasionally, some buffer components may crystallize that will dissolve at room temperature. Store stock solution at 4oC.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{o}C until the expiration date printed on the label. The whole kit stability is at least 6 months from the date of shipping under appropriate storage conditions. After opening the kit components, the shelf life is approx. 2 months.

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

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag.

1. Dilute **Wash buffer, DHT-HRP conjugate** before use.

2. Label or mark the microtiter well strips to be used on the plate. Pipet **50 µl** stds., controls, and samples into appropriate wells.

3. Pipet **100 µl** of Diluted DHT-HRP conjugate into **each well. Mix gently.** Cover the plate and incubate for **60 minutes** at room temperature.

4. Aspirate and wash the wells **3 times** with 300 µl of diluted wash buffer. 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 µl** of HRP-substrate soln. (TMB) into each well. Mix gently. Cover the plate and incubate for **10-30 minutes** at room temperature on a plate shaker until dark blue color develops in std A. The reaction can be stopped sooner or prolonged until desired color is obtained.

6. Stop the reaction by adding **50 µl** of stopping solution to **all** Mix gently. Blue color turns yellow. Measure the absorbance at 450 nm using an ELISA reader. Color is stable for at least one hr after stopping.

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 wells the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

DILUTION OF SAMPLES

Samples containing more than 2500 pg/ml DHT should be first diluted with the zero std and then run with the standards and control) as described in the assay procedure. The results obtained should be multiplied by the appropriate first dilution factor.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Draw the standard curve on semi-log graph paper by plotting net absorbance values of standards against appropriate DHT concentrations. Read off the DHT concentrations of the control and patient samples.

EXPECTED VALUES

It is expected that each lab determines its own normal range for the population it serves. The following values are given for reference purpose only.

Men:	250-990 pg/ml
Women	24-368 pg/ml (premenopausal) 10-181 pg/ml (postmenopausal)