

**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 Pregnlone	#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-1905

**Human 5 $\alpha$ -Androstane-3 $\alpha$ , 17 $\beta$ -diol Glucuronide (3 $\alpha$ -Diol G)**

**ELISA KIT Cat. No. 1905**

**For Quantitative Determination of 3 $\alpha$  Diol G  
In Human Serum**

*For In Vitro Research Use Only*



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Kit Contents: (reagents for 96 tests)

C o m p o n e n t s	
Rabbit Anti-3 $\alpha$ Diol G Antibody coated microwell strip plate (96 wells); #1906	1 Plate
3 $\alpha$ Diol G <b>Std. A</b> (0 ng/ml), 2 ml; #1907A	1 vial
3 $\alpha$ Diol G <b>Std. B</b> (0.25 ng/ml), 0.6 ml; #1907B	1 vial
3 $\alpha$ Diol G <b>Std. C</b> (1 ng/ml), 0.6 ml; #1907C	1 vial
3 $\alpha$ Diol G <b>Std. D</b> (3 ng/ml), 0.6 ml; #1907D	1 vial
3 $\alpha$ Diol G <b>Std. E</b> (10 ng/ml), 0.6 ml; #1907E	1 vial
3 $\alpha$ Diol G <b>Std. F</b> (50 ng/ml), 0.6 ml; #1907F	1 vial
3 $\alpha$ Diol G- <b>HRP Conjugate (50X)</b> ; 0.3 ml, Dilute 1:50 with assay buffer; #1908	1 vial
<b>Assay Buffer</b> , 15 ml; #1905-AB	1 bottle
3 $\alpha$ Diol G <b>Control Low</b> # 1905CL; 0.6 ml	1 vial
3 $\alpha$ Diol G <b>Control High</b> # 1905CH; 0.6 ml	1 vial
<b>see vial for lot sp. conc.</b>	
<b>Wash Buffer Conc. (10X)</b> , 50 ml, WB-10	
<b>HRP substrate (TMB) Solution</b> ; 16 ml; TMB-10	
<b>Stop solution</b> , 6 ml, ST-10	
Complete Instruction Manual, M1905	

ADI's 3 $\alpha$  Diol G ELISA kit provides direct measurement of 3 $\alpha$  Diol G in human serum.

## Introduction

5 $\alpha$ -Androstane-3 $\alpha$ , 17 $\beta$ -diol glucuronide is a C19 steroid and is either abbreviated as 3 $\alpha$  Diol G, 5 $\alpha$  Diol G or simply,  $\alpha$  Diol G. It is produced mainly as a metabolite of testosterone and dihydrotestosterone (DHT). It is largely produced in target peripheral tissues such as the skin, especially around hair follicles. The stimulation by large amounts of 3 $\alpha$  Diol G leads to excessive hair formation, notably where hair is not normally present in women. In recent years the interest in the measurement of this steroid has increased among clinical investigators studying women suffering from idiopathic hirsutism. Among the steroids known to be precursors for 3 $\alpha$  Diol G are dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEAS), dihydrotestosterone (DHT), androstenedione and testosterone. Only 3 $\alpha$  Diol G has been shown to increase with hirsutism and decrease with treatment. This correlation has also been demonstrated in patients with polycystic ovarian syndrome (PCO). 3 $\alpha$  Diol G determinations have therefore proved to be a useful indicator in a variety of ways including monitoring the progress of treatment of idiopathic hirsutism and women with PCO. Furthermore, diabetic patients (both men and women) under cyclosporine A therapy have shown increased 3 $\alpha$  Diol G levels, a side effect resulting in the appearance of hair in previously hairless areas.

## 2. PRECISION

**Intra-assay precision:** Three samples were assayed ten times each on the same calibrator curve. The results (in ng/ml) are tabulated below:

Sample	Mean	SD	CV%
1	0.87	0.07	7.8
2	6.86	0.49	7.2
3	21.26	1.29	6.0

**Inter-assay precision:** Three samples were assayed ten times over a period of four weeks. The results (in ng/ml) are tabulated below:

Sample	Mean	SD	CV%
1	0.98	0.10	10.4
2	7.05	0.46	6.5
3	20.92	2.26	10.8

## 3. ACCURACY/RECOVERY

Spiked samples were prepared by adding defined amounts of 3 $\alpha$  Diol G to three patient serum samples. The results (in ng/ml) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1 Unspiked	0.67	-	-
+0.5	1.07	1.17	91.4
+5.0	4.99	5.67	88.0
+15.0	12.66	15.67	80.8
2 Unspiked	1.83	-	-
+0.5	2.07	2.33	88.8
+5.0	6.18	6.83	90.5
+15.0	17.64	16.83	104.8
3 Unspiked	12.76	-	-
+0.5	15.32	13.26	115.5
+5.0	19.22	17.76	108.2
+15.0	22.68	27.76	81.7

## 3. LINEARITY

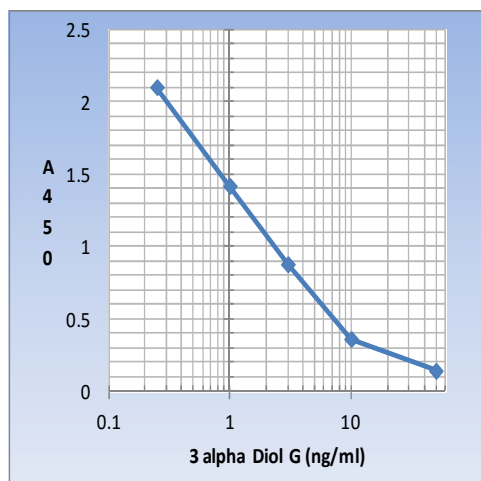
Three patient serum samples were diluted with calibrator A. The results (in ng/ml) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1	6.24	-	-
1:2	2.83	3.12	90.7
1:4	1.55	1.56	99.4
1:8	0.74	0.78	94.9
2	13.55	-	-
1:2	6.00	6.77	88.6
1:4	2.71	3.39	80.0
1:8	1.70	1.64	103.6
3	17.05	-	-
1:2	6.93	8.53	81.2
1:4	4.09	4.26	96.0
1:8	2.34	2.13	109.8

## WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples (ng/ml)	Net Mean $A_{450\text{ nm}}$
A1, A2	Std. A (0 ng/ml)	2.477
B1, B2	Std. B (0.25 ng/ml)	2.104
C1, C2	Std. C (1 ng/ml)	1.421
D1, D2	Std. D (3 ng/ml)	0.880
E1, E2	Std. E (10 ng/ml)	0.364
E1, E2	Std. F (50 ng/ml)	0.145

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-ADL\_ELISA

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

### 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 or 5-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.
5. If a sample reads more than 50 ng/ml then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

## PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabeled antigen (present in standards, controls and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed is inversely proportional to the concentration of 3 $\alpha$  Diol G in the sample. A set of standards is used to plot a standard curve from which the amount of 3 $\alpha$  Diol G in patient samples and controls can be directly read.

### MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (20-100  $\mu$ l) 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 3 $\alpha$  Diol G 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), H<sub>2</sub>SO<sub>4</sub> (stop solution), and Proclin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates). All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

### 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°C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

### REAGENT PREPARATION:

1. **Prepare 1x wash buffer** by diluting 10X stock (50 ml stock into 450 water).
2. **Prepare 1X 3 $\alpha$  Diol G HRP Conjugate:**  
Dilute stock conjugate 1:50 in assay buffer (20  $\mu$ l stock conjugate in 1 ml of assay buffer; prepare 11 ml for a full plate). Prepare 1x conjugate in required volume and do not store diluted conjugate for more than a few hours. All items must be at room temp prior to dispensing into the plate.

## STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°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. Repeated freezing and thawing is not recommended.

## 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. Pipet **50 µl** of standards, control, and serum samples into appropriate wells in *duplicate*.
2. Add 100 µl of 3α Diol G-HRP conjugate into each well. Mix gently. Cover the plate and incubate for **30 minutes** at room temperature on plate shaker (approx 200 rpm). If plate shaker is not available, plates can be mixed manually every 15-20 min.
3. Remove reaction mixture and **wash 3X** with 300 ul of 1X **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. Pipette **150 µl** of HRP-substrate solution (TMB). Mix gently. Cover the plate and incubate for **10-15 minutes** at room temperature on plate shaker. If plate shaker is not available, plates can be mixed manually. Blue color develops in standards and samples. Note: it is possible to vary the incubation time at this stage by 5 mins so as to get the maximum A450 in standard Zero =2.00-2.500.
5. Stop the reaction by adding **50 µl** of stopping solution to all wells. Mix gently. Blue color turns yellow. Measure the absorbance at 450 nm (620-630 refs filter can be used) 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°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.

## DILUTION OF SAMPLES and LIMITATIONS

It is recommended that each laboratory must determine its own normal and abnormal ranges. 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.

### 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.

Group	Range (ng/ml)
Males	1.53-14.82
Premenopausal	0.22-4.64
Postmenopausal	0.61-3.71
Puberty (Female)	0.51-4.03

### PERFORMANCE CHARACTERISTICS

#### Specificity:

The following compounds were tested for cross-reactivity with the Direct 3α Diol G ELISA kit with 3α Diol G cross-reacting at 100%.

Steroid	%Cross Reactivity
3α Diol G	100
Testosterone	0.2
Progesterone	0.16
Androstenedione	0.14
Cortisol	0.05

The following steroids were tested but cross-reacted at less than 0.01%: Corticosterone, Dehydroepiandrosterone, Dihydrotestosterone, Epiandrosterone, 17β-Estradiol and Estrone.

#### Sensitivity

The minimal detectable conc. of 3α Diol G is estimated to be **0.1 ng/ml**. 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.

### LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of 3α Diol G in human serum. The kit is not calibrated for the determination of 3α Diol G in saliva, plasma or 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. Only calibrator A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
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.