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-1865

Pregnenolone

ELISA KIT Cat. No. 1865, 96 Tests

For Quantitative Determination of Pregnenolone 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: Techsupport@4adi.com

Web Site: www.4adi.com

ELISA KIT Cat. No. 1865 (96 tests)

Kit Components (96 tests)	Cat #
Anti-Pregnenolone **Coated Strip plate**, (96 wells)	1866
Pregnenolone **Std. A**, 2 ml; 0 ng/ml	1867A
Pregnenolone **Std. B**, 0.5; 0.1 ng/ml	1867B
Pregnenolone **Std. C**, 0.5 ml; 0.4 ng/ml	1867C
Pregnenolone **Std. D**, 0.5 ml; 1.6 ng/ml	1867D
Pregnenolone **Std. E**, 0.5 ml; 6.4 ng/ml	1867E
Pregnenolone **Std. F**, 0.5 ml; 25.6 ng/ml	1867F
Pregnenolone **Low & High Controls** 0.5 ml	1867LC-HC
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 zing and thawing cycles.	
Pregnenolone- **HRP Conjugate (51X),** 300 µL	1869
Assay Buffer, 15 ml	1865-AB
Wash buffer 50 ml (10X)	W-10
TMB Substrate Solution, 16 ml	TMB-10
Stop Solution, 6 ml	T-10
Complete Instruction Manual	M-1865

Introduction

Pregnenolone (3β-hydroxypregn-5-en-20-one) is the first steroid to be derived from cholesterol in the pathway of steroidogenesis, and it is the common precursor for all of the adrenal and gonadal steroids. Its production occurs in the mitochondrion by cleavage of the C-20 side chain of cholesterol by the $P\text{-}450_{SCC}$ enzyme. Once produced, pregnenolone may be utilized by two pathways of steroidogenesis. Pregnenolone may either be converted to 17-OH pregnenolone via the enzymatic action of 17α-hydroxylase or to progesterone via the enzymatic action of 3β-hydroxysteroid dehydrogenase.

Elevated pregnenolone levels occur in forms of congenital adrenal hyperplasia (CAH), due to 3β-hydroxysteroid dehydrogenase deficiency or 17α-hydroxylase deficiencies. Higher levels have also been reported in women with idiopathic hirsutism. Studies on pregnenolone levels regarding sex and age differences indicate that maximum levels occur at approximately 17 and 16 years of age for women and men, while minimum levels occur at approximately 37 and 38 years of age for women and men, respectively. In general, women were found to have slightly higher values when compared to men.

Many areas of pregnenolone physiology remain to be investigated. Current research indicates that the determination of pregnenolone in serum may be useful for studying its metabolite, pregnenolone sulfate, which has been reported to have various effects in the mammalian brain and central nervous system.

INTRA-ASSAY PRECISION

Three samples (0.19, 1.04, 4.77 ng/ml) were assayed ten times each on the same calibrator curve. The values were SD 0.02, 0.85, 0.37 ng/ml and CV% 10.6, 12.3, 9.6% respectively.

INTER-ASSAY PRECISION

Three samples 0.22, 1.14, 4.56 ng/ml) were assayed ten times each on the same calibrator curve. The values were SD 0.03, 0.14, 0.44 ng/ml and CV% 14.5, 12.3, 9.6 respectively.

EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Males: N=30; mean 0.50 ng/ml Absolute range 0.1-3.4 ng/ml
Females: N=50; mean 0.55 ng/ml Absolute range 0.1-3.8 ng/ml

RECOVERY

Spiked samples were prepared by adding defined amounts of pregnenolone to four patient serum samples. The results (in ng/ml) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1 Unspiked	0.37	-	-
+4.14	5.31	4.51	117.7
2 Unspiked	0.77	-	-
+4.01	5.69	4.78	119.0
3 Unspiked	0.85	-	-
+3.98	5.18	4.83	107.2
4 Unspiked	1.47	-	-
+3.78	6.31	5.25	120.2

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	5.31	-	-
1:2	2.89	2.66	108.6
1:4	1.26	1.33	94.7
1:8	0.71	0.66	107.6
2	6.51	-	-
1:2	2.75	3.26	84.4
1:4	1.54	1.63	94.5
1:8	0.80	0.81	98.8
3	8.34	-	-
1:2	3.78	4.17	90.6
1:4	2.15	2.09	102.9
1:8	1.05	1.04	101.0

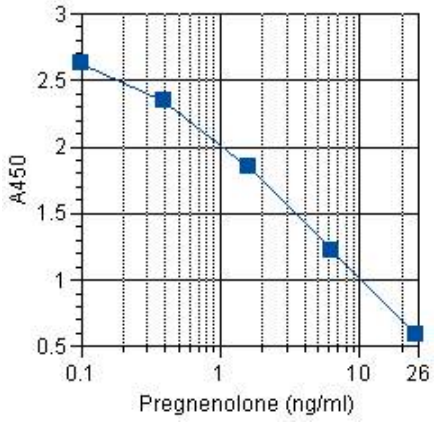
Species reactivity

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.). 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 (ng/ml)
A1, A2	**Std. A** (0 ng/ml)	2.850	
B1, B2	**Std. B** (0.1 ng/ml)	2.632	
C1, C2	**Std. C** (0.4 ng/ml)	2.347	
D1, D2	**Std. D** (1.6 ng/ml)	1.851	
E1, E2	**Std. E** (6.4 ng/ml)	1.217	
F1, F2	**Std. F** (25.6 ng/ml)	0.591	
F1, F2	**Sample 1**	1.441	4.0

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 Pregnenolone ELISA kit is **0.05 ng/ml.**

SPECIFICITY (CROSS REACTIVITY)

The following compounds were tested for cross-reactivity with the Direct Pregnenolone ELISA kit with pregnenolone cross-reacting at 100%.

Pregnenolone (100%), Progesterone (6%), Dehydroisoandrosterone (5.2%), 5α-Androstandiol (4.7%). Epiandrosterone, Pregnenolone Sulfate, Androstandione, 5α-Androsterone, DHEAS, Etiocholanolone were <1%.

The following steroids were tested but cross-reacted at less than 0.1%: Adrenosterone, Aldosterone, Androstenedione, Cholesterol, Corticosterone, 5α-DHT, 17β-Estradiol, Estriol and Testosterone.

PRINCIPLE OF THE TEST

Pregnenolone ELISA kit is based on competitive binding of human Pregnenolone from serum samples and enzyme-labeled Pregnenolone to Pregnenolone 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 Pregnenolone 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 Pregnenolone 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 Pregnenolone ELISA test is intended for *in vitro research* use only. The reagents contain proclin-300 as preservative. 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), H2SO4 (stop solution), and Prolcin-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 sera cannot be immediately assayed, these could be stored at -20^{o}C for up to six months. Avoid repeated zing and thawing of samples. No preservatives should be added to the serum.

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.

REAGENTS PREPARATION:

1. **Prepare 1X Wash buffer** in distilled water. Take 50 ml 10X concentrate and mix it with 450 ml distilled/deionized water. Store at 4oC.

2. **Prepare 1X solution of Pregnenolone-HRP conjugate**. Dilute 1:51 in assay buffer before use (e.g., **40 µL** of conjugate concentrate in 2 mL of assay buffer). If the whole plate is to be used dilute **240 µL** of concentrated conjugate in 12 mL of assay buffer. Prepare as needed and do not store 1x working solution for more than few hours.

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 HRP conjugate (1:51) with assay buffer and wash buffer (1:10) with water.

2. Pipet **50 µL of standards**, control, and serum samples into appropriate wells in *duplicate*.

3. Add **100 µL of HRP-conjugate working solution** into each well**.** Mix gently for 5-10 seconds. Cover the plate and incubate for **60 minutes at room temp**. with gentle shaking (~200 rpm). Failure to shake will decrease the total absorbance values.

4. Aspirate and **wash the wells 3 times** with 300 ul of 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 TMB substrate**. Mix gently for 5-10 seconds. Cover the plate and incubate for **15 minutes at room temp**.

6. Stop the reaction by adding **50 µL of stop solution** to all wells at the same timed intervals as in step 7. Mix gently for 5-10 seconds. Blue color turns yellow.

7. Measure the **A450 nm** using an ELISA reader within 20 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 pregnenolone in human serum. The kit is not calibrated for the determination of pregnenolone 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.

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.

5. If a sample reads more than 25.6 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