

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
#1870	Human Pregnenolone	#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-1870

**17-alpha HYDROXYPROGESTERONE
(17- α -OHP)**

ELISA KIT Cat. # 1870

**For Quantitative Determination of 17-alpha hydroxy
Progesterone In Human Serum**

For In Vitro Research Use Only



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

ELISA KIT Cat. No. 1870 (96 tests)

Kit Components(96 tests)	Cat #
Anti-17-alpha OHP Coated Strip plate , (96 wells)	1871
17-OHP Std. A , 10 ml; 0 ng/ml	1872A
17-OHP Std. B , 0.6 ml; 0.15 ng/ml	1872B
17-OHP Std. C , 0.6 ml; 0.5 ng/ml	1872C
17-OHP Std. D , 0.6 ml; 1.5 ng/ml	1872D
17-OHP Std. E , 0.6 ml; 3 ng/ml	1872E
17-OHP Std. F , 0.6 ml; 7.5 ng/ml	1872F
17-OHP Std. G , 0.6 ml; 20 ng/ml	1872G
17 OHP Control 1 , 0.6 ml	1873C1
17 OHP Control 2 , 0.6 ml	1873C2
All standards and controls are supplied in stabilized buffer containing proteins. 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.	
17-OHP-HRP Conjugate(100X), 300 ul	1874
Assay Buffer , ready to use, 20 ml	AS1870
Wash buffer 50 ml (10X)	WB-10
TMB Substrate Soln, 16 ml	TMB-10
Stop Solution, 6 ml	T-30
Complete Instruction Manual	M-1870

Introduction

The steroid 17 alpha-OH Progesterone is produced by the adrenal cortex and gonads. The 17 alpha-OH Progesterone has little progestational activity, but it is of intense clinical interest because it is the immediate precursor to 11-desoxycortisol (Cpd-S). Cpd-S is produced by 21-hydroxylation of 17 OHP, measurement of 17aOHP is a useful indirect indicator of 21-hydroxylase activity. In congenital 21-hydroxylase deficiency, the most common variety of congenital adrenal hyperplasia (CAH), 17 a OHP is secreted in abundant excess. Measurement of 17 alpha-OHP is therefore valuable in the initial diagnosis of CAH.

INTRA-ASSAY PRECISION

Three samples (1.91, 5.68, 9.38 ng/ml) were assayed ten times each on the same calibrator curve. The values were SD 0.096, 0.287, 0.602 and CV% 5.0, 5.1, 5.7% respectively.

INTER-ASSAY PRECISION

Three samples (1.69, 5.69, 9.38 ng/ml) were assayed ten times each on the same calibrator curve. The values were SD 0.116, 0.487, 0.464 ng/ml and CV% 6.8, 8.6, 4.9 respectively.

EXPECTED NORMAL VALUES

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

Group	Range (ng/ml)
Newborn	
5-30 day	0.7-2.5
31-60 day male	0.8-5.0
Children	0.5-2.3
3-14 yrs	0.07-1.7
Females	
Follicular phase	0.2-1.3
Luteal phase	1.0-4.5
Postmenopausal	0.2-0.9

RECOVERY

Spiked samples were prepared by adding defined amounts (0.15, 0.4, 1.6 ng/ml) of 17-OHP to three patient serum samples (1.82, 2.79, 3.7 ng/ml). Mean recoveries were 93-104%.

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

Sample	Obs.Result	Recovery%
1	3.52	-
1:2	1.50	86
1:4	0.88	101
1:8	0.46	107
2	5.47	-
1:2	2.7	99
1:4	1.21	89
1:8	0.66	97
3	18.29	-
1:2	8.75	96
1:4	4.54	99
1:8	2.27	100

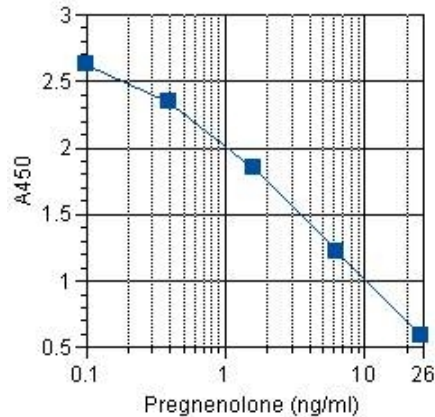
Species reactivity

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 most species, this kit should work in most species as long as the sample concn is within the range of this kit.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean $A_{450\text{ nm}}$	Calculated Conc (ng/ml)
A1, A2	Std. A (0 ng/ml)	2.544	
B1, B2	Std. B (0.15 ng/ml)	2.33	
C1, C2	Std. C (0.5 ng/ml)	1.76	
D1, D2	Std. D (1.5 ng/ml)	0.98	
E1, E2	Std. E (3 ng/ml)	0.70	
F1, F2	Std. F (7.5 ng/ml)	0.35	
G1, G2	Std. G (20 ng/ml)	0.199	
H1, H2	Sample	0.82	2.15

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 17-OHP ELISA kit is **0.11 ng/ml**.

SPECIFICITY (CROSS REACTIVITY)

The following compounds were tested for cross-reactivity with the Direct 17-OHP ELISA kit with 17-OHP cross-reacting at 100%.

17-OHP (100%), Progesterone (0.25%), 11-deoxycortisol (0.7%), DHEA, corticosterone, cholesterol, pregnenolone, pregnenolone sulfate, prednisone were <0.1%.

PRINCIPLE OF THE TEST

17-OHP (17 alpha-hydroxy progesterone) ELISA kit is based on competitive binding of human 17-OHP from serum samples and enzyme-labeled 17-OHP to 17-OHP 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 17-OHP 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 17-OHP 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 17-OHP ELISA test is intended for *in vitro* research use only. The reagents contain proclin-300 as preservative. Some components of the kit (stds/controls) may be 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 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 sera cannot be immediately assayed, these could be stored at -20°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°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°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 4°C.
2. **Prepare 1X HRP-conjugate.** Dilute **1:100 in assay buffer** (e.g., 20 ul in 2 ml assay buffer for 1 strip or prepare 150 ul in 15 ml buffer for a full plate assay). Prepare as needed and do not store 1x working conjugate.

TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE 20-25°C BEFORE USE).

1. Label or mark the microtiter well strips to be used on the plate. Dilute wash buffer (1:10) with water.
2. Pipet **50 ul of standards**, control, and serum samples into appropriate wells in *duplicate*.
3. Add **150 ul of 17-OHP-HRP conjugate** 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. If a shaker is not available it is possible to manually mix the plates every 5-10 minutes for 5 secs.
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 ul TMB substrate.** Mix gently for 5-10 seconds. Cover the plate and incubate for **15 minutes at room temp on a plate shaker.** Blue color develops in standards and samples. Incubation time may be adjusted by 5-10 min so as to achieve the Std.A A450 ~2.0-3.00.
6. Stop the reaction by adding **50 ul of stop solution** to all wells at the same timed intervals as in step 5. 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°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 17-OHP in human serum. The kit is not calibrated for the determination of 17-OHP 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