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	**#1955**	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		

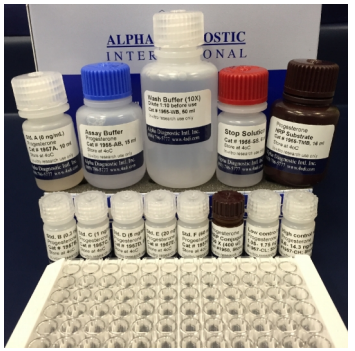
#3000	Human Rheumatoid Factors IgM (RF)
#3100	Human anti-dsDNA
#3200	Anti-Nuclear Antibodies (ANA)

Instruction Manual No. M-1955

PROGESTERONE

ELISA KIT Cat. No. 1955

For Quantitative Determination of Progesterone In Human Serum



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ALPHA DIAGNOSTIC INTERNATIONAL

India Contact:

Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034
Ph: +91-11-42208000, 42208111, 42208222

Mobile: +91-9810521400
Fax: +91-11-42208444
Email: customerservice@atzlabs.com
Web: www.atzlabs.com

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

PROGESTERONE ELISA KIT Cat. No. 1955

For Quantitative Determination of Progesterone In Serum

Kit Contents: (reagents for 96 tests)

Components	Cat. #
Anti-Progesterone IgG Coated microwell strip plate (96 wells), Ready-to-use	1956
Progesterone **Std. A** (0 ng/ml), 10 ml	1957A
Progesterone **Std. B** (0.3 ng/ml), 0.5 ml	1957B
Progesterone **Std. C** (1 ng/ml), 0.5 ml	1957C
Progesterone **Std. D** (5 ng/ml), 0.5 ml	1957D
Progesterone **Std. E** (20 ng/ml), 0.5 ml	1957E
Progesterone **Std. F** (60 ng/ml), 0.5 ml	1957F
Progesterone **Control serum low (#1957-CL) & high (1957-CH)** (exact values printed on vials)	
Progesterone -HRP Conjugate (100X), 0.4 ml (dilute 1:100 with assay buffer)	1958
Assay Buffer (ready to use), 15 ml	1955-AB
Wash Buffer Conc (10X), 50 ml	1955-WB
HRP substrate Solution; 16 ml	1955-TMB
Stop solution, 6 ml	1955-SS
Complete Instruction Manual	M-1955

Introduction

Progesterone (pregn-4-ene-3,20-dione) is a C21 steroid hormone. The molecular weight of this steroid hormone is 314.5. Progesterone is a female sex hormone, which, in conjunction with estrogens, regulates the accessory organs during the menstrual cycle, and it is particularly important in preparing the endometrium for the implantation of the blastocyte and in maintaining pregnancy. In non-pregnant women progesterone is mainly secreted by the corpus luteum whereas in pregnancy the placenta becomes the major source. Minor sources are the adrenal cortex for both sexes and testes for males.

Progesterone circulates in blood mainly bound to Corticosteroid Binding Globulin(CBG), Sex Hormone Binding Globulin (SHBG) and Albumin. Only 2-10% of the total concentration circulates as free hormone. The measurement of plasma progesterone is clinically used to confirm ovulation and normal function of the corpus luteum in non-pregnant women. Abnormal progesterone secretion has been implicated in premenstrual tension, irregular shedding of endometrium, dysmenorrhoea, and luteal insufficiency.

ADI's Progesterone ELISA kit provides for the measurement of progesterone in human serum. This kit can be adapted to measure progesterone in animals samples

PERFORMANCE CHARACTERISTICS

1. Sensitivity

The lower detection limit is 0.1 ng/ml.

2. PRECISION

Intra-assay precision:

	Pool A	Pool b	Pool c
N	24	24	24
Mean (ng/ml)	0.5	4.9	17.0
C.V. (%)	10.8	6.4	6.5

Inter-assay precision:

	Pool A	Pool b	Pool c
N	10	10	10
Mean (ng/ml)	0.6	5.3	15.7
C.V. (%)	11.7	10.9	6.8

3. ACCURACY

Recovery studies were performed by mixing an aliquot of poled serum and progesterone standard. The progesterone values were measured and % of recovery was determined.

Initial Values (ng/ml) (200 ul)	Conc. spiked (ng/ml) (100 ul)	Expected values (ng/ml)	Observed values (ng/ml)	Recovery (%)
5.0	10	3.6	4.0	111
5.0	10.0	6.7	6.5	97
9.5	5.0	8.0	9.5	118
9.5	50.0	23.0	21.0	93

4. Specificity

The following compounds were tested for crossreactivity of the assay.

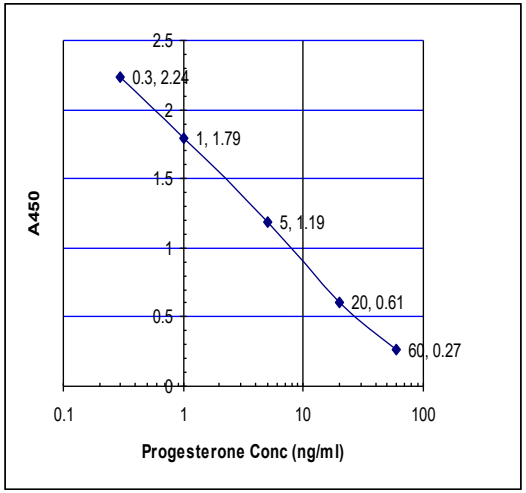
Compounds	% crossreactivity
Progesterone	100
11-alpha-hydroxyprogesterone	100
11-Deoxycorticosterone	1.5
17-hydroxyprogesterone	0.7
Pregnelone	0.15
Testosterone	<0.1
Estradiol	<0.1
Estriol	<0.1
Cortisol	<0.1

References: Kakabakos SE (1992) Clin Chem. 38, 725; Cameron EDH (1973) Clin. Chem. 19, 1403; Elder PA (1987) Clin. Chim. Acta 162, 199

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples (ng/ml)	Net Mean $A_{450\ nm}$	Calculated Conc. (ng/ml)
A1, A2	**Std. A** (0)	2.37	
B1, B2	**Std. B** (0.3 ng/ml)	2.24	
C1, C2	**Std. C** (1 ng/ml)	1.79	
D1, D2	**Std. D** (5 ng/ml)	1.19	
E1, E2	**Std. E** (20 ng/ml)	0.61	
F1, F2	**Std. G** (60 ng/ml)	0.28	
G1, G2	**Sample 1**	0.73	15.4

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)

Animal (Species crossreactivity)

Progesterone 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. Our human progesterone kit has been used in mouse serum (see refs below).

Yata A 2009 Hum. Reprod., Jul 2009; 24: 1748 - 1753.
Yang Q 2006 Endocrinology. 2006 Oct;147(10):4772-80. used mouseserum

PRINCIPLE OF THE TEST

Progesterone ELISA kit is based upon competitive solid phase ELISA. Progesterone in the sample competes with enzyme-linked progesterone for a fixed and limited number of antibody binding sites on the coated plates. In this solid-phase system, the antibody bound progesterone will remain on the well while unbound progesterone will be removed by washing. A color (blue) is developed when the substrate, TMB is mixed with the antibody bound progesterone-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 progesterone in the sample.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (20-100 μl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The Alpha Diagnostic International Progesterone ELISA test is intended for *in vitro research* use only. The reagents contain proclin-300 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), 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 freezing and thawing of samples. No preservatives should be added to the serum. Azide or merthiolate may interfere with the assay.

REAGENTS PREPARATION

Before use, **dilute wash buffer (1:10)** with distilled water. Diluted buffer can be stored at 4oC for several weeks.

Dilute Progesterone-HRP conjugate stock 1:100 with assay buffer. Need 100 ul/well. Prepare 12 ml for a full plate (120 ul in 12 ml assay buffer or 10 ul per ml).

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. Reconstituted control serum is stable for one week at 2-8oC. Repeated freezing and thawing is not recommended.

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

1. **Dilute wash buffer (1:10) with distilled water** (50 ml stock in total of 500 ml).

2. Pipet **25 ul of standards**, control, and serum samples into appropriate wells in *duplicate*.

3. Add **100 ul of HRP conjugate** into **each well.** Mix gently for 5-10 seconds. Cover the plate and incubate for **60 minutes at room temperature (25-30oC) on a plate shaker (200 rpm).** If a shaker is not available, plates can be manually shacked for 5-10 secs a few times.

4. Remove reaction mixture and **wash 3X with 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. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing. **Note**: Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay

5. Dispense **150 ul TMB substrate per well**. Mix the plate gently for 5-10 seconds. Cover the plate and incubate at room temp. for **15 minutes on a plate shaker (200 rpm).** If a shaker is not available, plates can be manually shacked for 5-10 secs a few times**.** Blue color develops in standards and positive wells. **Note**: The incubation time can be varied +5 min to achieve calibrator A values of about 2.50.

6. Stop the reaction by adding **50 ul of stop solution** to all wells. Mix gently for 5 seconds. Measure the A450 nm using an ELISA reader within 30 min. **Note**: If A450 values of calibrator exceeds the readable range of the ELISA reader (>2.000), the plate can be read at 405nm or 415nm filter. The optical density will be lower but it will not impact the standards or sample values.

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 index (Y) on the semi-log paper. Draw a point-to-point line through the mean of the duplicate point. If a immunoassay software is being used, a 4-parameter or 5-pararmeter curve is recommended.

2. Obtain the value of sample progesterone 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.

3. If a sample reads more than 60 ng/ml then dilute it with calibrator A at a dilution of no more than 1:8. The concentration obtained must be multiplied by the dilution factor.

DILUTION OF SAMPLES and LIMITATIONS

It is recommended that each laboratory must determine its own normal and abnormal ranges. Extrapolation of progesterone values beyond the standard curve may yield variable results. Samples containing >60 ng/ml progesterone can be diluted with 0 standard and retested. Calibrators and controls from other manufacturers may contain serum preservatives 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.

EXPECTED VALUE

1. It is recommended that each laboratory should determine its own normal and abnormal range. Serum samples from 30 normal men were assayed, and 99% of the values were found to be less than 1.2 ng/ml. The values from 30 normal women serum were from 0.75-20 ng/ml.

2. the following values can be used as preliminary guidelines until the laboratory establishes its own normal values.

	Progesterone (ng/ml)	
WOMEN	Follicular phase	0.2-1.4
	Luteal phase	4-25
	Menopause	0.1-1.0
MEN		0.1-1.2