

*Instruction Manual No. M-1855*

## **Progesterone (Saliva)**

**For Quantitative Determination of Progesterone  
In **Saliva****

*For In Vitro Research Use Only*

**ELISA KIT Cat. No. 1855 (96 tests)**

For Quantitative Determination of Progesterone in Human Saliva

Kit Components(96 tests)	Cat #
Anti-Progesterone Coated Strip plate, (96 wells)	1856
Progesterone <b>Std. A</b> , 10 ml; 0 pg/ml	1857A
Progesterone <b>Std. B</b> , 0.5; 20 pg/ml	1857B
Progesterone <b>Std. C</b> , 0.5 ml; 100 pg/ml	1857C
Progesterone <b>Std. D</b> , 0.5 ml; 500 pg/ml	1857D
Progesterone <b>Std. E</b> , 0.5 ml; 2000 pg/ml	1857E
Progesterone <b>Std. F</b> , 0.5 ml; 5000 pg/ml	1857F
Progesterone <b>Control</b> 0.5 ml	1867G
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.	
Assay buffer, 15 ml	1858
Progesterone-HRP Conjugate, 300 ul (100X)	1859
Wash buffer 50 ml (10X)	W-10
TMB Substrate Soln, 16 ml	TMB-10
Stop Solution, 6 ml	T-10
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**Introduction**

Progesterone is a C-21 female sex steroid hormone with a variety of physiological effects. In the follicular phase of the menstrual cycle, progesterone is produced in low levels. It increases to the LH peak and then sharply rises 3 to 4 days later to higher levels, remaining elevated through the 10th to 12th days after the LH peak. Next there is a sharp decline to the low levels of the follicular phase. Progesterone is responsible for the induction of the cyclic changes in the endometrium of the uterus allowing implantation and successful growth of the fertilized ovum and maintenance of pregnancy.

Progesterone measurements are useful in documenting ovulation and in the management of difficulties during the first trimester of pregnancy. Levels of progesterone may be useful in the evaluation of sterility due to luteal phase defects, prediction of impending abortion, and the diagnosis of ectopic pregnancy. Drugs such as, oral contraceptives, superovulatory drugs, estrogen replacement therapy medication, and GnRH analogues may affect normal values of progesterone. The removal of ovarian function following surgical oophorectomy or chemotherapy may influence salivary progesterone values. The determination of salivary progesterone combines a highly sensitive technique and non-invasive sample collection that is of value in clinical and research studies.

ADI progesterone saliva has been tested for human saliva. It may be adapted for other species. Separate kit serum/plasma progesterone is also available.

**INTRA-ASSAY PRECISION**

Three samples were assayed ten times each on the same calibrator curve. The results (in pg/ml) are tabulated below:

Sample	Mean	SD	CV%
1	32.93	4.39	13.3
2	78.73	4.63	5.9
3	302.67	22.30	7.37

**INTER-ASSAY PRECISION**

Three samples were assayed ten times over a period of four weeks. The results (in pg/ml) are tabulated below:

Sample	Mean	SD	CV%
1	30.83	3.90	12.7
2	75.03	7.73	7.7
3	241.06	26.23	10.9

**RECOVERY**

Spiked samples were prepared by adding defined amounts of progesterone to two patient saliva samples (1:1). The results (in pg/ml) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1 Unspiked	4.38	-	-
+ 100	58.93	52.19	112.9
+ 500	240.57	252.19	95.4
+ 2000	851.70	1002.19	85.0
2 Unspiked	7.49	-	-
+ 100	46.27	53.75	86.1
+ 2000	894.58	1003.75	89.1
+ 5000	2694.49	2503.75	107.6

**LINEARITY**

Three patient saliva samples were diluted with calibrator A. The results are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1	1005.66	-	-
1:2	473.10	502.83	94.1
1:4	218.29	251.41	86.8
1:8	115.63	125.71	92.0
2	1462.5	-	-
1:2	700.48	731.25	95.8
1:4	327.69	365.62	89.6
1:8	172.12	182.81	94.1
3	2279.9	-	-
1:2	1061.0	1139.95	93.1
1:4	497.67	569.98	87.3
1:8	239.59	284.99	84.1

**REFERENCE VALUES**

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values in females. Follicular Phase (<100 pg/ml), Luteal Phase (100-500 pg/ml), Postmenopausal (<50 pg/ml).

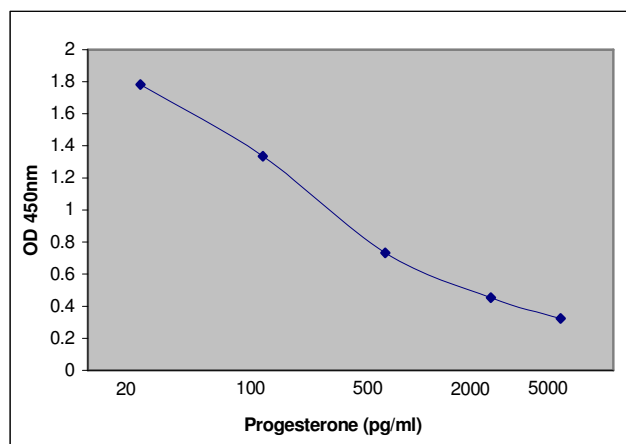
**Species reactivity**

This kit has been designed and tested for human saliva 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 conc. is within the range of this kit.

## WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean $A_{450\text{ nm}}$	Calculated Conc'n (pg/ml)
A1, A2	<b>Std. A</b> (0 pg/ml)	2.850	
B1, B2	<b>Std. B</b> (20 pg/ml)	2.632	
C1, C2	<b>Std. C</b> (100 pg/ml)	2.347	
D1, D2	<b>Std. D</b> (500 pg/ml)	1.851	
E1, E2	<b>Std. E</b> (2000 pg/ml)	1.217	
F1, F2	<b>Std. F</b> (5000 pg/ml)	0.591	
F1, F2	<b>Sample 1</b>	0.917	300

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 Progesterone saliva ELISA kit is **20 pg/ml**.

### SPECIFICITY (CROSS REACTIVITY)

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

Progesterone (100%), 11-alpha-OH-Progesterone (100%), Deoxycorticosterone (1.7%), 17-OH-Progesterone, 5 $\alpha$ -Androstan-3 $\beta$ , 17 $\beta$ -diol, Corticosterone, Pregnenolone, (<0.4%).

The following steroids were tested but cross-reacted at less than 0.1%: Cortisol, Cortisone, Danazol, DHEAS, Estradiol, 5 $\beta$ -Pregnan-3 $\alpha$ , 17 $\alpha$ , 21 $\alpha$ -triol-20-one, 5 $\beta$ -Pregnan-3 $\alpha$ , 17-diol-20-one, Pregnan-3 $\alpha$ , 20 $\alpha$ -diol and Testosterone.

## PRINCIPLE OF THE TEST

Progesterone ELISA kit is based on competitive binding of human Progesterone from saliva samples and enzyme-labeled Progesterone to Progesterone 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 Progesterone 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 Progesterone in samples and control is read off the standard curve.

## MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (20-100 ul) and multichannel pipet; Reagent troughs, plate shaker (orbital shaker), plate washer (recommended) and ELISA plate Reader.

## PRECAUTIONS

The Progesterone ELISA test is intended for *in vitro* research use only. The reagents contain 0.1% Prolcin-300 and care should be taken when disposing solutions. The Controls may prepared from human fluids have 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.

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 Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

## SPECIMEN COLLECTION AND HANDLING

Approximately 1 ml of saliva is required per duplicate determination. Collect 4-5 ml of saliva into a clean glass tube\* between 7-10 am without force or inducement and before eating, drinking or brushing the teeth. Simply rinse the mouth with water before collection. Do not use blood-contaminated specimens. Store samples at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. \*Do not use cotton or polyester rolls or plastic collection tubes for collecting saliva samples in this assay, since it has been well established that false elevated results will occur.

## SPECIMEN PRETREATMENT

Specimen tubes are to be placed into a freezer and allowed to freeze. When ready to use, the specimens are to be thawed, heated at 60°C for 1 hour, and then centrifuged. The supernatants are to be collected and poured into freshly labelled tubes. Do not use blood-contaminated specimens. If samples are to be used at a later date store frozen.

## REAGENTS PREPARATION

Dilute **Wash buffer** :1:10 in distilled water.

Prepare **1X** solution of **Progesterone-HRP conjugate**. Dilute 20 ul stock conjugate in 2 ml of assay buffer. (120 ul in 12 ml for complete 96-well 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. The unused portions of the standards should be stored at 2-8°C or stored frozen in small aliquots.

## 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 enzyme conjugate (1:100) with assay buffer and wash buffer (1:10) with water.
2. **Specimen pretreatment:** Freezing, Heating at 60°C for 1 Hour & Centrifugation
3. Pipet **50 ul of standards**, control, and saliva samples into appropriate wells in *duplicate*.
4. Add **100 ul of diluted enzyme conjugate** into each well. Mix gently. Cover the plate and incubate on an **orbital or ELISA plate shaker** for **60 minutes** at room temp (25-28°C). Failure to shake will decrease the total absorbance values.
5. Aspirate and **wash the wells 3 times** with 300 ul of running tap water or distilled water. 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.
6. Add **150 ul TMB substrate**. Mix gently. Cover the plate and incubate for **15 minutes** at room temp (Zero calibrator should be ~1.5-2.0). It is possible to increase or decrease the incubation time by a few minutes if necessary.
7. Stop the reaction by adding **50 ul of stop solution** to all wells at the same timed intervals as in step 6. Mix gently.
8. Measure the **absorbance at 450 nm** using an ELISA reader within 30 min. If the OD exceeds the upper limit of detection or if a 450nm filter is unavailable, a 405 or 415nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples

## 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 progesterone in human saliva. The kit is not calibrated for the determination of progesterone in serum, plasma or other specimens of human or animal origin.
2. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
3. Only calibrator A may be used to dilute any high saliva samples. The use of any other reagent may lead to false results.
4. 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.

## EXTRACTION VS. NON-EXTRACTION COMPARITIVE STUDY

The Progesterone Saliva ELISA method was validated by the following comparative study between:

1. Prior extraction of saliva samples with diethyl acetate.
2. Prior heating of saliva samples for 1 hour at 60-70 °C.

The data from these 22 random saliva samples show a strong correlation of  $r=0.91$ . As a result, the heating method was chosen due to its easier and less time consuming technique.

## 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 5000 pg/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