ASSAY PROCEDURE

Sample Pretreatment: **Freezing, Heating at 60°C for 1 Hour & Centrifugation.**

All reagents must reach room temperature before use. Calibrators, controls and samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Prepare working solutions of the progesterone-HRP conjugate and wash buffer.
2. Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette 50 µL of each calibrator, control and sample into correspondingly labelled wells in duplicate.
4. Pipette 100 µL of the conjugate working solution into each well. (We recommend using a multichannel pipette.)
5. Incubate on a plate shaker (approximately 200 rpm) for 1 hour at room temperature.
6. Wash the wells 3 times with 300 µL of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended.)
7. Pipette 150 µL of TMB substrate into each well at timed intervals.
8. Incubate on a plate shaker for 10–20 minutes at room temperature (or until calibrator A attains dark blue colour for desired OD).
9. Pipette 50 µL of stopping solution into each well at the same timed intervals as in step 7.
10. Read the plate on a microplate reader at 450 nm within 20 minutes after addition of the stopping solution.

* If the OD exceeds the upper limit of detection or if a 450 nm filter is unavailable, a 405 or 415 nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of samples or controls.

CALCULATIONS

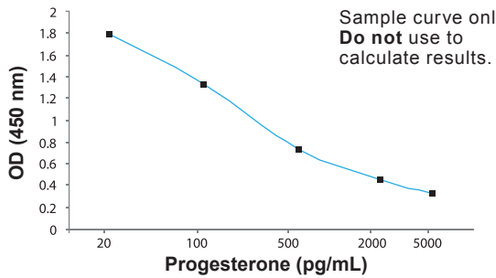
1. Calculate the mean optical density of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
3. 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 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.

TYPICAL TABULATED DATA

Sample data only. **Do not** use to calculate results.

Calibrator	OD 1	OD 2	Mean OD	Value (pg/mL)
A	2.252	2.320	2.286	0
B	1.795	1.768	1.782	20
C	1.352	1.322	1.337	100
D	0.730	0.736	0.733	500
E	0.453	0.451	0.452	2000
F	0.341	0.307	0.324	5000
Unknown	0.915	0.919	0.917	300

TYPICAL CALIBRATOR CURVE



Sample curve only. **Do not** use to calculate results.

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 Progesterone Saliva ELISA kit with progesterone cross-reacting at 100%.

Steroid	% Cross Reactivity
Progesterone	100
11α-OH-Progesterone	100
Deoxycorticosterone	1.7
17-OH-Progesterone	0.4
5α-Androstane-3β, 17β-diol	0.3
Corticosterone	0.3
Pregnenolone	0.2

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

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 defi ned amounts of progesterone to two 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 saliva samples were diluted with calibrator A. The results (in pg/mL) 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

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.

REFERENCES

1. Finn MM, et al. Normal Salivary Progesterone Levels Throughout the Ovarian Cycle as Determined by a Direct Enzyme Immunoassay. *Fertil Steril*. 1988; 50(6): 882–7.
2. Walker RF, et al. Characterization of Profiles of Salivary Progesterone During the Luteal Phase of Fertile and Subfertile Women. J Endocrinol. 1985; 104(3):441–6.
3. Zorn JR, et al. Salivary Progesterone as an Index of the Luteal Function. *Fertil Steril*. 1984; 41(2):248–53.
4. Vining RF, et al. Hormones in Saliva: Mode of Entry and Consequent Implications for Clinical Interpretation. *Clin Chem*. 1983; 29(10):1752–6.
5. Lenton EA, et al. Measurement of Progesterone in Saliva: Assessment of the Normal Fertile Range Using Spontaneous Conception Cycles. *Clin Endocrinol (Oxf)*. 1988; 28(6):637–46.
6. Heasley RN, Thompson W. Salivary Progesterone Measurements in the Normal Menstrual Cycle. *Ir J Med Sci*. 1986; 155:(1)19–22.
7. Check JH, et al. Falsely Elevated Steroidal Assay Levels Related to Heterophile Antibodies Against Various Animal Species. *Gynecol Obstet Invest*. 1995; 40(2):139–40.

SYMBOLS

CE European Comformity
IVD In vitro diagnostic device
Consult instructions for use
Contains sufficient for <n> tests
Storage Temperature
Legal Manufacturer
Use by
REF Catalogue Number
EC REP Authorized representative
LOT Lot number
X # Dilute 1: # Before use