Instructions for Use

Progesterone Saliva ELISA

Enzyme-linked immunoassay for the quantitative determination of progesterone in human saliva.

REF **RE52281**

Σ **96**

2-8°C

EU: IVD CE

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1. INTENDED USE

Enzyme immunoassay for the quantitative determination of progesterone in human saliva.

2. SUMMARY AND EXPLANATION

Progesterone, a C21-steroid, is a female sex hormone and a precursor in the metabolism of other steroids.
Progesterone is synthesized mainly in the corpus luteum of the ovaries, during the main part of pregnancy in the placenta and in very small amounts for the production of other steroids in the adrenal cortex and in the testes. At the latter locations progesterone is important for the synthesis of aldosterone, cortisol, testosterone and 17-β-estradiol.
In the circulation the main part of progesterone is bound to the corticoid binding globulin (CBG, Transcortin), to the sex hormone binding globulin (SHBG) and to albumin. 1 – 2 % of progesterone circulates as a free hormone in plasma. Only this portion represents the active part in the endocrine regulation. The free hormone is released in equal amounts in saliva. An enzymatic metabolization of portions of this hormone in the saliva glands is presumed.
Progesterone is one important hormone of the endocrine regulation of the menstrual cycle. After ovulation progesterone is secreted by the corpus luteum which develops from the ovulated follicle in the ovaries. The progesterone level rises in the 6^{th} – 8^{th} day after ovulation to a plateau. Together with estradiol it inhibits the release of LH and FSH in the pituitary gland by a negative feedback mechanism. Progesterone is secreted into the circulation in a pulsating way. Because of the lysis of the corpus luteum, the progesterone level decreases in the last 3 days of the cycle to a pre-ovulatory level.
In pregnancy, beginning at the 8^{th} gestation week, the placenta becomes the major source of progesterone production during the 2^{nd} and 3^{rd} trimester.
The course of progesterone levels in the circulation is reflected in its concentration in saliva.
The most important task of progesterone is to prepare the genital organs of the women for a potential implantation and to maintain the pregnancy. The main effects of progesterone are to introduce the secretory phase of the endometrium, to suppress the contractions of the uterus and to stimulate the growth of mammary tissue, as well as other effects on the metabolism and the endocrine system in women.
Regarding physiology, the measurement of progesterone in saliva is useful in monitoring the menstrual cycle in women in order to determine the time of ovulation and to assess the function of the corpus luteum, which becomes important in the early stages of pregnancy. Because the fluctuations of the progesterone levels depends also on individual situations, it is very convenient to get a hormone profile by repeatedly collecting saliva samples.

3. TEST PRINCIPLE

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the competition principle. An unknown amount of antigen present in the sample and a fixed amount of enzyme labelled antigen compete for the binding sites of the antibodies coated onto the wells. After incubation the wells are washed to stop the competition reaction. After the substrate reaction the intensity of the developed colour is inversely proportional to the amount of the antigen in the sample. Results of samples can be determined directly using the standard curve.

4. WARNINGS AND PRECAUTIONS

1. For *in-vitro diagnostic* use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. The cleaning staff should be guided by the professionals regarding potential hazards and handling.
9. Some reagents contain ProClin 300 as preservative. In case of contact with eyes or skin, flush immediately with water. When disposing reagents, flush with a large volume of water to avoid built-up.

5. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8 °C. Keep away from heat or direct sunlight. The storage and stability of specimens and prepared reagents is stated in the corresponding chapters.

The microtiter strips are stable up to the indicated expiry after the kit is broken. Make sure that the broken bag is tightly closed when stored at 2-8 °C.

6. SPECIMEN COLLECTION AND STORAGE

Saliva

<table>
<tr><td colspan="4">The patient should not eat, drink, chew gums or brush teeth for 30 min before sampling. Otherwise rinse mouth thoroughly with cold water 5 min prior to sample collection. Do not collect samples when oral diseases, inflammation or lesions exist (blood contamination).
Saliva can be collected in a suitable sampling device. A minimum of 0.5 mL liquid should be collected. It is recommended to freeze samples at –20°C prior to laboratory testing. After thawing, mix and centrifuge 10 min at 2000 – 3000 x g to remove particulate material.</td></tr>
<tr><td>  </td><td colspan="3">Take care that the saliva samples are visually acceptable.
(Reddish color indicating blood contamination)</td></tr>
<tr><td>  </td><td colspan="3">Do not use any PE (Polyethylene) devices or Salivettes for sampling; this in most cases will result in significant interferences. Glas tubes can be used as well, but in this case special attention is necessary for excluding any interference caused by the stopper.</td></tr>
<tr><td>Storage:</td><td>18-25°C</td><td>2-8°C</td><td>≤ -20°C (Aliquots)</td></tr>
<tr><td>Stability:</td><td>> 2 weeks</td><td>> 4 weeks</td><td>≥ 6 months</td></tr>
</table>

7. MATERIALS SUPPLIED

Quantity	Symbol	Component
1 x 12x8	MTP	**Microtiter Plate** Break apart strips. Coated with antibodies against Progesterone (monoclonal, mouse)
1 x 6 x 1 mL	CAL A-F	**Standard A-F** Ready to use. 0; 25; 50; 250; 1000; 5000 pg/mL Contains: Progesterone, stabilizers, <0.1% ProClin 300.
1 x 2 x 1 mL	CONTROL 1+2	**Control 1+2** Ready to use. Contains: Progesterone, stabilizers, <0.1% ProClin 300. Exact concentrations see vial labels or QC certficate.
1 x 13 mL	ENZCONJ	**Enzyme Conjugate** Ready to use. Contains: Progesterone conjugated to HRP, stabilizers.
1 x 100 mL	WASHBUF CONC	**Wash Buffer** Concentrate (10x) Contains: phosphate buffer, Tween.
1 x 15 mL	TMB SUBS	**TMB Substrate Solution** Ready to use. Contains: TMB, Buffer, stabilizers
1 x 15 mL	TMB STOP	**TMB Stop Solution** Ready to use. 1 M H_2SO_4

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volume: 50; 100 µL
2. A suitable sampling device should be used.
3. Vortex mixer
4. Disposable glass tubes (for sample dilution)
5. 8-Channel Micropipettor with reagent reservoirs
6. Wash bottle, automated or semi-automated microtiter plate washing system
7. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
8. Bidistilled or deionised water
9. Paper towels, pipette tips and timer

9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
5. Use a pipetting scheme to verify an appropriate plate layout.
6. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
7. Microtiter plate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microtiter plate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
8. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

10. PRE-TEST SETUP INSTRUCTIONS

⚠	The contents of the kit for 96 determinations can be divided into 3 separate runs. **The volumes stated below are for one run with 4 strips (32 determinations).**

10.1. Preparation of concentrated components

Dilute / dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
30 mL	**WASHBUF** **CONC**	ad 300 mL	bidist. water	1:10	Mix vigorously.	2-8°C	4 weeks

10.2. Dilution of Samples

Samples suspected to contain concentrations higher than the highest standard have to be diluted with Sample Diluent (available from IBL REF KLZZ731). **Note: Dilution has to be made in glass tubes.** Measured results must be multiplied with the dilution factor to obtain corrected results.

11. TEST PROCEDURE

1.	Pipette **50 µL** of each **Standard, Control and sample** into the respective wells of the microtiter plate.
2.	Pipette **100 µL Enzyme Conjugate** into each well. **Thoroughly mix for 10 seconds.**
3.	**Incubate 60 min at 18-25°C.**
4.	Discard incubation solution. Wash plate **3 x** with **250 µL** of diluted **Wash Buffer.** Remove excess solution by tapping the inverted plate on a paper towel.
5.	Pipette **100 µL** of **TMB Substrate Solution** into each well.
6.	**Incubate 30 min** at **18-25°C**.
7.	Stop the substrate reaction by adding **100 µL** of **TMB Stop Solution** into each well. Shake plate briefly. Color changes from blue to yellow.
8.	**Measure** optical density with a photometer at **450 nm** (Reference-wavelength: 600–650 nm) **within 15 min** after pipetting of the Stop Solution.

12. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or comparable standards/laws. User and/or laboratory must have a validated system to get diagnosis according to GLP. All kit controls must be found within the acceptable ranges as stated on the labels and the QC certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. It is recommended to participate at appropriate quality assessment trials.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

13. CALCULATION OF RESULTS

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logistics or Logit-Log.

For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

The concentration of the samples can be read directly from the standard curve.

In case of diluted samples the values have to be multiplied with the corresponding dilution factor.

Samples showing concentrations above the highest standard have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and reassayed.

Saliva samples with remarkably elevated values should be reviewed for blood contamination.

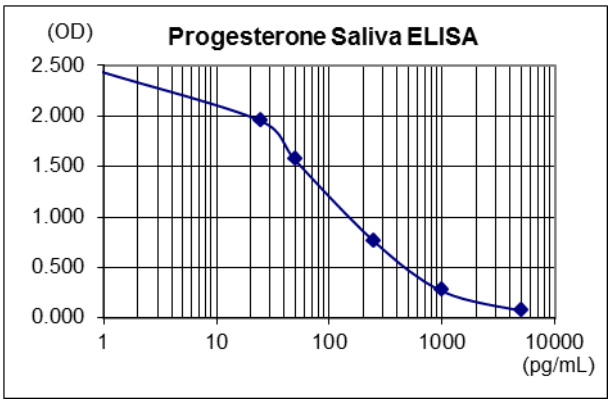
Conversion:

Progesterone (pg/mL) x 3.17 = pmol/L

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	Progesterone (pg/mL)	OD_{Mean}	OD/OD_{max} (%)
A	0	2.447	100.0
B	25	1.948	80
C	50	1.560	64
D	250	0.757	31
E	1000	0.265	11
F	5000	0.072	3



14. EXPECTED VALUES

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.

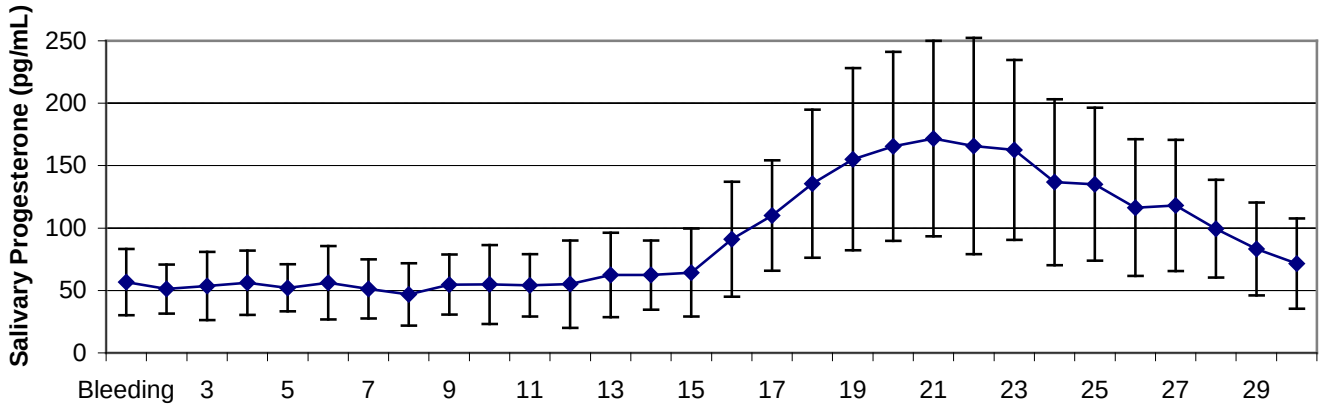
Apparently healthy subjects show the following values:

	Progesterone (pg/mL)		
♀	Premenopausal (n = 27 monthprofiles)	Follicular Phase	28-82 pg/mL
		Luteal Phase	127-446 pg/mL
	Postmenopausal (n = 6)		18-51 pg/mL
♂	n = 49		< 51 pg/mL

It is recommended that each laboratory establishes its own range of normal values.

To establish a normal range for this test, a study was performed with pre-menopausal women to collect saliva samples three times per day (morning, midday and afternoon). The 3 samples were pooled and the progesterone concentration measured to obtain a daily value throughout the menstrual cycle. It had been observed that some women may have periodic atypical profiles. 4 women in this study exhibited atypical progesterone profiles. The following chart shows the results of the study.

Menstrual cycle of 27 women (age: 19-43 years) without contraceptiva



15. LIMITATIONS OF THE PROCEDURE

Specimen collection and storage have a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details.

For cross-reactivities, see PERFORMANCE.

16. PERFORMANCE

Analytical Specificity (Cross Reactivity)	**Substance**		**Cross Reactivity (%)**		**Cross-reactivity of other substances tested ≤ 0.001%**	
	Pregnenolone		0.042		17α- Hydroxypregnenolone, Corticosterone, Cortisol, Cortisone, DHEA, Estradiol, Estriole, Estrone	
	Desoxycorticosterone		0.029			
	Androstenedione		0.022			
	Testosterone		0.020			
	21-Deoxycortisol		0.010			
	17α- Hydroxyprogesterone		0.004			
Analytical Sensitivity (Limit of Detection)	3.13 pg/mL	Mean signal (Zero-Standard) - 2SD				
Functional Sensitivity	3.10 pg/mL	Mean Conc. < 20 % CV				
Precision		Range (pg/mL)	CV (%)	Mean (%)	Samples	n
	Intra-Assay	31 - 2837	3.8 – 6.6	4.9	4	20
	Inter-Assay	21 - 1582	4.4 – 9.6	6.7	4	10
	Inter-Lot	22 - 1560	5.4 – 9.0	7.3	4	10
Linearity	Range (%)	Mean (%)	n	Serial dilution up to		
	94 -110	103	4	1:16		
Recovery	Range (%)	Mean (%)	n	Added concentration		
	102 -111	106	4	100 – 4000 pg/mL		
Method Comparison	IBL-ELISA = 0.9306 x IBL-Luminescence IA - 4.5355			$r^2 = 0.94$; r = 0.97; n = 118		

17. PRODUCT LITERATURE REFERENCES

1. Gray S. et al. 2010, Salivary Progesterone Levels Before Menarche: A Prospective Study of Adolescent Girls. J Clin Endocrinol Metab, 95(7):3507–3511
2. Kumari V. et al. 2010, Evidence for a Role of Progesterone in Menstrual Cycle-Related Variability in Prepulse Inhibition in Healthy Young Women. Neuropsychopharmacology 35:929–937
3. Schoofs D, Wolf O. 2011. Are salivary gonadal steroid concentrations influenced by acute psychosocial stress? A study using the Trier Social Stress Test (TSST). International Journal of Psychophysiology 80:36–43
4. Lewis, J.G. 2006. Steroid Analysis in Saliva: An overview. Clin Biochem Rev Vol 27 139-146
5. Wood, P. 2009. Salivary steroid assays – research or routine? Ann Clin Biochem 46: 183–196

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER'S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.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