Instructions for Use

Progesterone
Luminescence Immunoassay

Luminescence immunoassay for the *in-vitro-diagnostic* quantitative determination of progesterone in human saliva.

REF	RE62021	RE62029
Σ	96	960

2-8 °C

EU: IVD CE U.S.: *For in-vitro diagnostic use only.*

I B L I N T E R N A T I O N A L G M B H

Flughafenstrasse 52a
D-22335 Hamburg, Germany

Phone: +49 (0)40-53 28 91-0
Fax: +49 (0)40-53 28 91-11

IBL@IBL-International.com
www.IBL-International.com

1. INTENDED USE

Luminescence immunoassay for the *in-vitro-diagnostic* quantitative determination of active free progesterone (a female hormone) in human saliva. Measurements obtained by this device may be used in the diagnosis and treatment of disorders of the ovaries and can be used as an aid for confirmation of ovulation.

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

Luminescence immunoassay 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 addition of the luminescence substrate solution the intensity of the luminescence measured 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. Some reagents contain sodium azide (NaN_3) as preservatives. In case of contact with eyes or skin, flush immediately with water. NaN_3 may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with a large volume of water to avoid azide build-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 sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters.

The microtiter strips are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2–8 °C.

6. SPECIMEN COLLECTION AND STORAGE

Saliva

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. Saliva flow can be stimulated by chewing on a piece of Parafilm®. 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.

⚠ **Take care that the saliva samples are visually okay.**
(Reddish color indicating blood contamination)

Storage:	37 °C	18-25 °C	2-8 °C	≤ -20 °C (Aliquots)
Stability:	1 week	> 2 weeks	> 4 weeks	≥ 6 months

7. MATERIALS SUPPLIED

Quantity RE62021	Quantity RE62029	Symbol	Component
1 x 12x8	10 x 12x8	MTP	**Microtiter Plate** Coated with rabbit anti-mouse antibody.
1 x 0.15 mL	1 x 1.5 mL	ENZCONJ CONC	**Enzyme Conjugate Concentrate (101x)** Contains: alkaline phosphatase conjugate, 0.1 % NaN_3.
1 x 9 mL	1 x 90 mL	ANTISERUM	**Progesterone Antiserum** Red colored. Ready to use. Contains: anti-Progesterone antibodies (mouse).
1 x 10 mL	1 x 100 mL	ASSAYBUF	**Assay Buffer** Red colored. Ready to use. Contains: Tris buffer, BSA, 0.1 % NaN_3.
7 x 1.0 mL	7 x 3.5 mL	CAL A-G	**Standard A-G** 0; 10; 25; 50; 100; 300; 1000 pg/mL Ready to use. Contains: Progesterone, Buffer, BSA, 0.1 % NaN_3.
2 x 1.0 mL	2 x 3.5 mL	CONTROL 1+2	**Control 1+2** Ready to use. Concentrations / acceptable ranges see QC Certificate.
1 x 9 mL	1 x 90 mL	LUMINREAG AP	**Chemiluminescence Reagent AP** Ready to use. Contains: acridan based substrate.
1 x 100 mL	5 x 100 mL	WASHBUF CONC	**Wash Buffer Concentrate (10x)** Contains: Tris buffer, Tween, NaN_3.
3 x	12 x	FOIL	**Adhesive Foil**

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volume: 20; 50; 1000 µL
2. A suitable sampling device should be used (can be ordered separately from IBL under REF RE69991)
3. Orbital shaker (400-600 rpm)
4. Vortex mixer
5. 8-Channel Micropipettor with reagent reservoirs
6. Wash bottle, automated or semi-automated microtiter plate washing system
7. Luminescence Immunoassay-Reader (e.g. Berthold microtiter plate luminometer)
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. Some components contain ≤ 250 µL solution. Take care that the solution is completely on the bottom of the vial before opening.
5. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
6. Use a pipetting scheme to verify an appropriate plate layout.
7. 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.
8. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate 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.
9. 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 lyophilized or concentrated components

Dilute / dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
10 mL	WASHBUF CONC	ad 100 mL	bidist. water	1:10	Mix vigorously.	2-8 °C	4 weeks
20 µL	ENZCONJ CONC	with 2 mL	ASSAYBUF	1:101	Mix without foaming.	Prepare freshly and use only once.	

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 under REF KLZZ731). 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 **20 µL** of each **Standard, Control and sample** into the respective wells of the microtiter plate.
2.	Pipette **50 µL** of freshly prepared **Enzyme Conjugate** into each well.
3.	Pipette **50 µL** of **Progesterone Antiserum** into each well. Cover plate with adhesive foil.
4.	**Incubate 4 h** at **RT (18-25 °C)** on an orbital shaker (400-600 rpm).
5.	Remove adhesive foil. Discard incubation solution. Wash plate **4 x** with **250 µL** of diluted **Wash Buffer.** Remove excess solution by tapping the inverted plate on a paper towel.
6.	Pipette **50 µL** of **Chemiluminescence Reagent AP** into each well in same time delay and order as Luminometer later will measure (Berthold Luminometer for e.g. needs 2 sec per well).
7.	**Measure** relative luminescence units with a luminometer after **10 min.**

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 other applicable standards/laws. 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 RLU 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 Logisitcs 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:

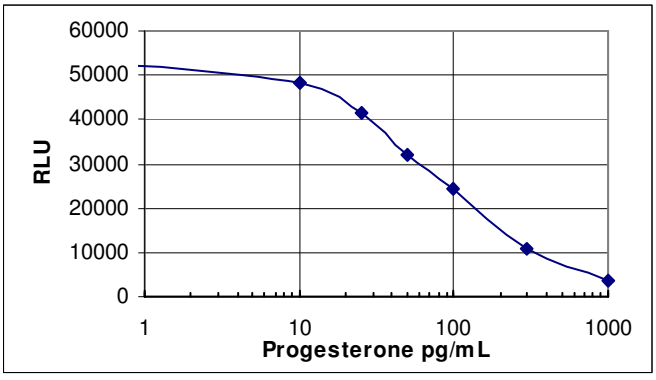
Progesterone (pg/mL) x 3.17 = pmol/L

Reportable Ranges:
Saliva: 2.6–1000 pg/mL Progesterone

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	Progesterone (pg/mL)	Mean RLU	RLU / RLU_{max} (%)
A	0	54860	100
B	10	48396	88
C	25	41301	75
D	50	32101	59
E	100	24198	44
F	300	10626	19
G	1000	3587	7



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.

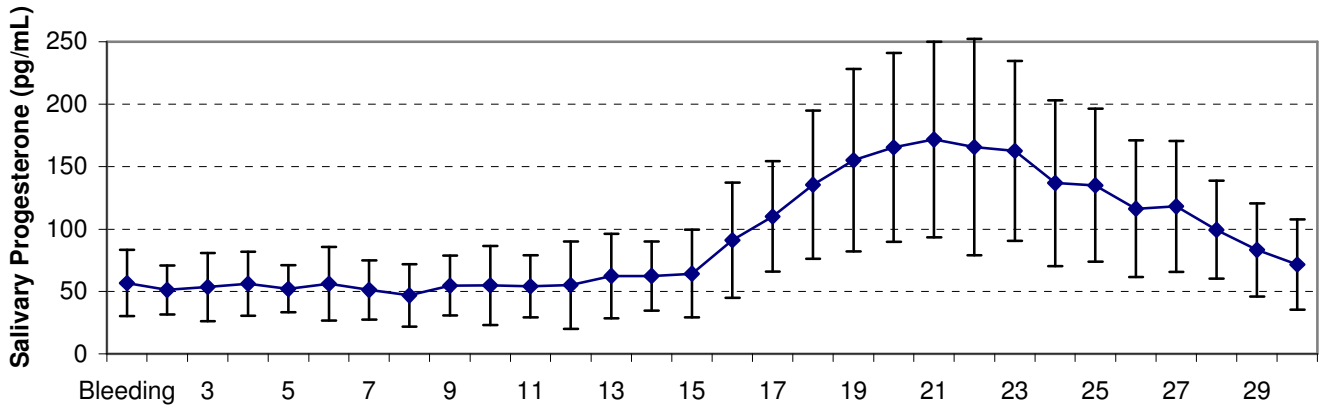
Apparently healthy subjects show the following values:

	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 has a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details.

For cross-reactivities, see PERFORMANCE.

Blood contamination of more than 0.25 % in saliva samples will affect results, and usually can be seen by eye.

Concentrations of NaN_3 > 1.0 % and thimerosal > 0.25 % interfere in this assay and may lead to false results.

16. PERFORMANCE

Analytical Specificity (Cross Reactivity)

Substance	Cross Reactivity (%)
17α-OH-Progesterone	1.84
6α-Methyl-17α-OH-Progesterone	1.41
Pregnenolone	0.41
Desoxy-Corticosterone	0.28
Androsterone Sulfate	0.25
Androstenedione	0.20
Androsterone	0.20
DHEA-S	0.11
Corticosterone	0.06

Cross-reactivity of other substances tested ≤ 0.1 %

Analytical Sensitivity (Limit of Detection)	2.6 pg/mL	Mean signal (Zero-Standard) – 2 SD
Functional Sensitivity	8.0 pg/mL	Mean conc. < 20 % CV

Precision	Mean (pg/mL)	SD	CV (%)	Mean (pg/mL)	SD	CV (%)
Intra-Assay (10x)	11.54	0.69	6.0	24.7	0.69	2.8
	50.2	1.53	3.1	100.0	5.02	5.0
	218.0	5.1	2.3	822.1	5.9	0.7
Inter-Assay (10x)	10.6	1.99	18.8	21.6	3.0	13.9
	76.5	5.8	7.5	245.7	16.1	6.6
	504.7	42.6	8.4	817.1	40.9	5.0

Linearity	Dilution	Measured (pg/mL)	Recovery (%)	Dilution	Measured (pg/mL)	Recovery (%)
	-	337.8	100	-	116.0	100
	1:2	155.6	92	1:2	59.2	102
	1:4	65.6	78	1:4	26.5	91
	1:8	35.1	83	1:8	16.3	112
	1:16	17.5	83	1:16	7.5	103
	1:32	11.4	108			
	-	779.2	100	-	1282.4	100
	1:2	349.5	90	1:2	773.4	120
	1:4	173.9	89	1:4	394.5	102
	1:8	82.6	85	1:8	209.3	108
	1:16	40.4	83	1:16	115.6	120
	1:32	20.9	86	1:32	52.3	108
				1:64	21.7	90

Recovery	Added (pg/mL)	Measured (pg/mL)	Recovery (%)	Measured (pg/mL)	Recovery (%)
	0	27.1	100	62.5	100
	10	37.1	102	68.2	94
	25	59.3	114	86.4	99
	50	93.6	121	109.5	97
	100	151.7	119	149.3	92
	300	361.1	110	355.6	98
	1000	1174.8	114	1044.9	98
	0	101.9	100	199.5	100
	10	112.9	101	220.3	105
	25	128.1	101	246.9	110
	50	146.7	97	274.4	110
	100	198.0	98	323.5	108
	300	418.7	104	564.6	113
	1000	1100.9	100	1497.3	125

Method Comparison versus RIA	IBL-Assay = 0.89 x RIA + 25.5 pg/mL r^2 = 0.94; n = 97	RIA Range = 0–916 pg/mL LUM Range = 2–945 pg/mL

17. PRODUCT LITERATURE REFERENCES

1. Lewis, J.G. 2006. Steroid Analysis in Saliva: An overview. Clin Biochem Rev Vol 27 139-146
2. 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.

IBL AFFILIATES WORLDWIDE

IBL International GmbH Flughafenstr. 52A, 22335 Hamburg, Germany	Tel.: E-MAIL: WEB:	+ 49 (0) 40 532891 -0 Fax: -11 IBL@IBL-International.com http://www.IBL-International.com
IBL International Corp. 194 Wildcat Road, Toronto, Ontario M3J 2N5, Canada	Tel.: E-MAIL: WEB:	+1 (416) 645 -1703 Fax: -1704 Sales@IBL-International.com http://www.IBL-International.com

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