Instructions for Use

17beta-Estradiol Saliva Luminescence Immunoassay

Luminescence immunoassay for the in-vitro-diagnostic quantitative determination of 17beta-estradiol in human saliva.

REF **RE62141 / RE62149**

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 measurement of 17beta-Estradiol in human saliva. Results obtained by this device may be used in the diagnosis and treatment of various hormonal sexual disorders. This test is not intended for assessing placental function in complicated pregnancy.

2. SUMMARY AND EXPLANATION

17β-Estradiol [1, 3, 5 (10) -estratriene-3, 17β-diol; E2] is a C18 steroid hormone and the most important natural estrogen. It is present in females and males. In the former, the estrogens stimulate the growth of sex organs and the development of secondary sexual characteristics, and they also affect the gonadotropin secretion.

In the males, the role of 17β-Estradiol is less well defined although it seems to be involved in the regulation of gonadotropin secretion. In non-pregnant women 17β-Estradiol is almost exclusively produced by ovary. Especially after having changed into the menopause, estrogenes are produced by the liver, the brain, the muscles and by the adipose tissue, too. Moreover, the measurement of 17β-Estradiol is helpful to determine a lack of estrogenes, which may be expressed as delayed puberty, primary and secondary amenorrhoea, and occurs in the menopause. Other hormone assays are required for proper interpretation and differential diagnosis. The concentration of gonadotropin should be measured to find the origin of the lack of estrogenes (synthesis or regulation). The determination of 17β-Estradiol is useful for the diagnosis of *pubertas praecox* of girls. Concerning men, the level of 17β-Estradiol may be used for the differential diagnosis of gynecomastia.

Being principally bound to sex hormone binding globuline (SHBG) and to serum albumin, only 1 - 3 % of estradiol circulating in plasma is present in its free form. Only this portion represents the active part in the endocrine regulation. The free hormone is released in equal amounts in saliva.

Because the fluctuations of the 17β-Estradiol 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. The cleaning staff should be guided by the professionals regarding potential hazards and handling.
9. 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 sunlight. The storage and stability of specimens and prepared reagents is stated in the corresponding chapters.

The microtiter strips are stable up to 6 w 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 acceptable. (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 RE62141	Quantity RE62149	Symbol	Component
1 x 12x8	10 x 12x8	**MTP**	**Microtiter Plate** Coated with donkey anti-sheep antibody.
1 x 150 µL	1 x 1.5 mL	**ENZCONJ** **CONC**	**Enzyme Conjugate** Concentrate (101x) Contains: alkaline phosphatase conjugate, NaN_3.
1 x 9 mL	1 x 90 mL	**ANTISERUM**	**Estradiol Antiserum** Red colored. Ready to use. Contains: anti-17β-Estradiol antibodies (sheep), NaN_3
1 x 10 mL	1 x 100 mL	**ASSAYBUF**	**Assay Buffer** Red colored. Ready to use. Contains: Tris buffer, BSA, NaN_3.
7 x 1.0 mL	7 x 3.5 mL	**CAL A-G**	**Standard A-G** 0.0; 0.9; 2.0; 4.0; 8.0; 16; 64 pg/mL Ready to use. Contains: 17β-Estradiol, Buffer, ProClin 300
2 x 1.0 mL	2 x 3.5 mL	**CONTROL 1+2**	**Control 1+2** Ready to use. Contains: 17β-Estradiol, Buffer, ProClin 300. Exact concentrations see vial labels or QC certficate.
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; 100; 1000 µL
2. A suitable sampling device should be used
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
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. 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.
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 **50 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 **Estradiol-Antiserum** into each well.
4.	Cover plate with adhesive foil. **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 (RLU) 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 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 mean 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 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:

17β-Estradiol (pg/mL) x 3.67 = pmol/L

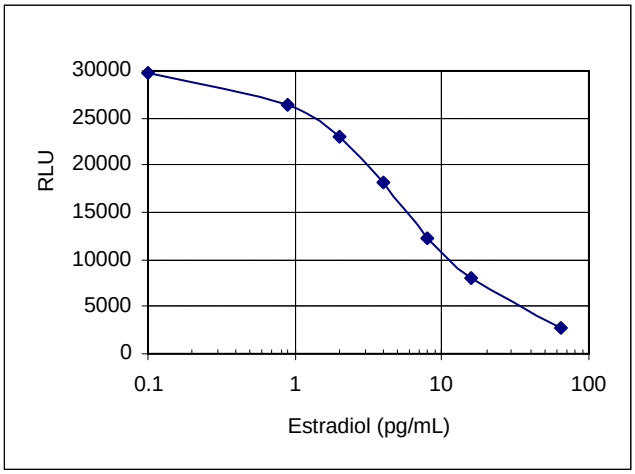
Reportable Ranges:

Saliva: 0.3 – 64 pg/mL 17β-Estradiol

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	17β-Estradiol (pg/mL)	RLU_{Mean}	RLU/RLU_{max} (%)
A	0.0	29799	100
B	0.9	26383	89
C	2.0	23032	77
D	4.0	18133	61
E	8.0	12333	41
F	16	7927	27
G	64	2744	9



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.

Published ranges of „normal“ males and females tend to vary slightly depending on the method used for assessment.

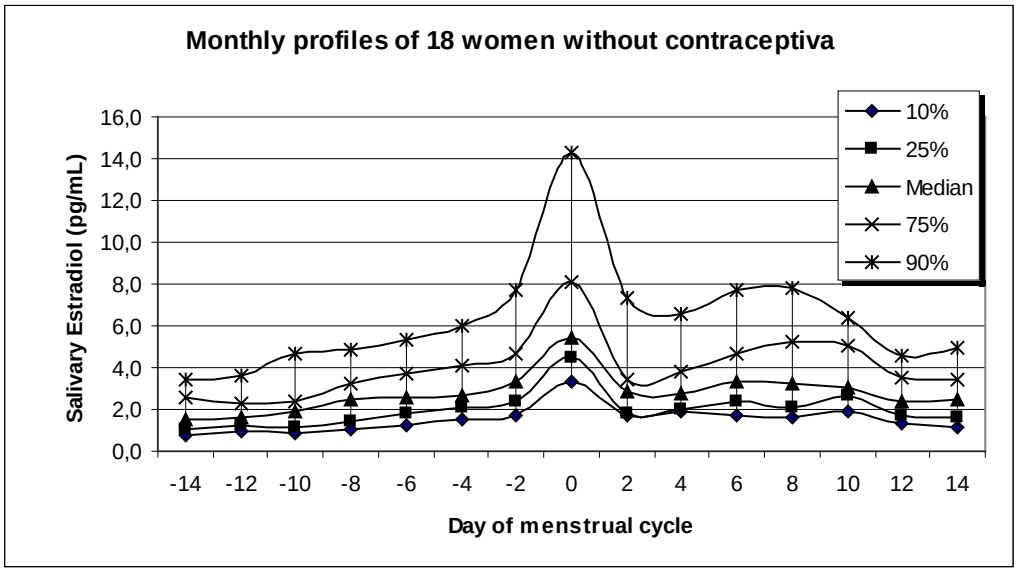
Apparently healthy subjects show the following values:

Estradiol (pg/mL)				
♀	Premenopausal	n = 18, Age: 19-43 monthprofiles (~1500 single samples)	Follicular Phase	0.8 - 7.7
			Midcycle	3.4 - 14.3
			Luteal Phase	1.1 - 7.8
	Postmenopausal	n = 13, Age 50-61		< 4.3
♂		n = 40, Age: 20-63	-	0.4 - 3.3

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

To establish a normal range in saliva for this test, a study was performed with pre-menopausal women not using contraceptives. Saliva samples were collected three times per day (morning, midday, and afternoon/evening). The three samples were pooled and the estradiol concentration measured to obtain a daily value throughout the menstrual cycle.

The following chart shows the results of the study:



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.

The17beta-Estradiol Saliva Luminescence Immunoassay should not be used for patients being treated with the drug Fulvestrant (Faslodex®) which cross reacts in the 17beta-Estradiol Saliva Luminescence Immunoassay and could lead to falsely elevated test results.

The following blood components do not have a significant effect (+/-20% of expected) on the test results up to the below stated concentrations:

Saliva		
	Conc.	**Estradiol (pg/mL)**
Blood	0.125%	1.4; 7.3
Thimerosal	0.1%	11; 80
NaN_3	1%	11; 80

16. PERFORMANCE

Analytical Specificity (Cross Reactivity)	Substance		Cross-reactivity (%)		Cross-reactivity of other substances tested ≤ 0.01%
	17β-Estradiol		100		
	Estrone		14.0		
	Deoxycortisol		0.58		
	Estriole		0.50		
	Fulvestrant		0.42		
	Estrone-3-Sulfate		0.26		
	Ethinylestradiol		0.05		
	Estradiol-Glucuronide		0.03		
	Epiestradiol		0.02		
	Dihydrotestosterone		0.02		
Analytical Sensitivity (Limit of Detection)	Saliva	0.3 pg/mL	Mean signal (Zero-Standard) - 2SD		
Functional Sensitivity	Saliva	0.9 pg/mL	Mean Conc. < 20% CV		
Precision	Mean (pg/mL)	SD (pg/mL)	CV (%)	n	
Intra-Assay	1.6	0.2	13.3	20	
	11.6	1.1	9.4	20	
	39.6	2.8	7.2	20	
Inter-Assay	3.0	0.4	14.8	20	
	10.4	0.8	7.2	20	
	33.6	4.0	11.9	20	

	Conc. (pg/mL)	Dilution	Measured (pg/mL)	Recovery (%)
Linearity	Saliva 1 (46.2)	-	46.2	100
		1:2	24.4	106
		1:4	12.8	111
		1:8	6.4	111
	Saliva 2 (52.6)	-	52.6	100
		1:2	25.8	98
		1:4	13.1	100
		1:8	6.1	92
		1:16	3.2	96
	Saliva 3 (62.6)	-	62.6	100
		1:2	32.0	102
		1:4	17.9	114
		1:8	9.0	115
		1:16	3.6	92

	Conc. (pg/mL)	Added (pg/mL)	Measured (pg/mL)	Recovery (%)
Recovery	Saliva 1 (1.2)	0.9	2.1	102
		2	3.0	95
		4	4.7	91
		8	8.0	87
		16	17.3	101
		64	53.6	82
	Saliva 2 (4.8)	0.9	5.6	98
		2	6.1	90
		4	7.4	83
		8	11.7	91
		16	17.4	84
		64	62.2	90
	Saliva 3 (21.4)	0.9	22.6	101
		2	22.1	94
		4	24.2	95
		8	29.0	99
		16	34.2	91
Method Comparison versus ELISA	IBL 17beta-Estradiol Saliva Luminescence Immunoassay = 1.02 x ELISA + 0.24			r^2 = 0.94 r = 0.97 n = 39

17. PRODUCT LITERATURE REFERENCES

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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.


IBL International GmbH
Flughafenstr. 52A, 22335 Hamburg, Germany

Tel.: + 49 (0) 40 532891 -0 Fax: -11
E-MAIL: IBL@IBL-International.com
WEB: http://www.IBL-International.com