Instructions for Use

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17beta-Estradiol Saliva ELISA

Enzyme immunoassay for the quantitative determination of 17beta-Estradiol in human saliva.

REF **30121045**

Σ **96**

2°C 8°C

EU: IVD CE 2797 U.S.: *For in-vitro diagnostic use only.*

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REVISION HISTORY OF INSTRUCTIONS FOR USE

Changes from the previous version 2019-10 to actual version 2023-02	
Cover page	Layout change
Chapter 2	Additional chapter
Chapter 3	Update to scientific validity
Chapter 5	Additional information
Chapter 6	Update
Chapter 7	Update and additional data
Chapter 8	Update and additional data
Chapter 13	Update and additional data
Chapter 15	Update and additional data
Chapter 16	Update and additional data
Chapter 17	Layout change and additional data
Chapter 18	Update
Chapter 19	Update
Symbol page	Layout change

1. INTENDED USE

Enzyme immunoassay for the quantitative determination of 17beta-Estradiol in human saliva.

2. INTENDED PURPOSE

The 17-beta-Estradiol Saliva ELISA is intended for the measurement of 17-beta-Estradiol in human saliva. Estradiol is the most important natural estrogen and present in both women and men. The measurement of the hormone level is useful for detection of estrogen deficiency. Estradiol has an impact on puberty, primary and secondary amenorrhea and menopause. This test is not intended for assessing placental function in complicated pregnancy. Additional hormone assays are recommended for an interpretation of the estradiol level.

The 17-beta-Estradiol Saliva ELISA is based on the competition principle and measured on an absorbance reader. The assay is semi-automated requiring general purpose laboratory instruments and consumables such as absorbance microplate reader/washer, vortexer and pipettes to execute the test. Test results may be calculated manually from a standard curve and compared to laboratory established reference ranges from healthy adults (i.e. normal ranges). The device can be adapted to different ELISA processors and can be applied to open automated platforms like EVOlyzer.

The test kit is intended for professional laboratory use by trained personnel. The test kit is not for home or layperson use. The test kit is intended for manual use.

3. SUMMARY AND EXPLANATION

17beta-Estradiol [1, 3, 5 (10) -estratriene-3, 17beta-diol; E2] is a C18 steroid hormone and an important natural estrogen, which derives from the precursor cholesterol and belongs to the group steroid hormones called estrogens, which is present in women and men.[1; 2] Estradiol is the major estrogen in the body involving different functions within the human physiology throughout life.[3] Estradiol, the most potent bioactive estrogen, is primarily synthesized from testosterone in the ovarian follicles in females, whereas in males it is produced by the testes and extraglandular conversion of androgens.[4; 5] Estradiol is synthesized from testosterone or estrone, a derivative of cholesterol.[6]

The main hormones responsible for advancing secondary sexual characteristics are two androgens: testosterone and dehydroepiandrosterone (DHEA), which facilitate masculine development, and estradiol, an estrogen which facilitates feminine development.[7] Estradiol is the primary estrogen released from the gonads and other peripheral tissues.[8]

Levels of 17beta-Estradiol secretion increased with pubertal progression. Estradiol is an important hormone present in both women and men. Estrogen deficiency in boys and in girls but especially in young girls it has an impact on puberty. Girls with delayed puberty show advancing pubertal maturation when administered estradiol. The measurement of estradiol levels is useful regarding puberty to investigate if early puberty (precocious puberty) occurs in girls. Estradiol is 4 - 9 times higher in late adolescent girls as compared to childhood.[9] Ovarian volumes increase with pubertal breast stage and correlate positively with circulating estradiol levels.[10]

Amenorrhea is classified into primary and secondary amenorrhea. Primary amenorrhea is defined as the absence of menarche by the age of 14 years, while secondary amenorrhea describes the absence of menses for more than six months in previously menstruating women.[11] In older women estrogens level

should be observed in regards to menopause.[12] Additionally, estradiol plays a role in ovulation, menstrual cycle and menopause and in this regard the function of ovaries and placenta.[13-17]

In addition to its role as a natural hormone, estradiol is used in the realm of hormone replacement therapy during menopause.[18]

Naturally estradiol levels both in women and men decline with age, however, the estradiol level in older men are higher than in postmenopausal women and serve different functions in the male body (bone maturation and sex interest). Estradiol levels are related to body fat mass, bone health, affect skin metabolism and sex interest in men. Estradiol levels in men are higher than in postmenopausal women and comparable to estradiol levels in the early follicular phase. It is also worth mentioning that a correlation between testosterone and estradiol levels exists due to testosterone being the major precursor of estradiol.[19]

Additional parameters should be measured for an interpretation of the results e.g. DHEA or testosterone.[7] Other physiological aspects where estradiol is involved are amenorrhea (primary and secondary) and menopause, which are indicated by low estradiol levels.[11; 21]

In serum steroid hormones are bound to sex hormone binding globulin (SHBG) and to serum albumin, only 1 - 3 % of estradiol circulates in plasma and is present in its free form. Only this portion represents the bioactive form of the endocrine regulation. However, in saliva only the free hormone is present as the protein bound hormones cannot pass the membranes into the oral cavity.

A good correlation between hormones in saliva with the free fraction in serum.[20] Proportions of estradiol levels in saliva are similar, between 0.2 % and 7.9 % of total serum concentrations.[22] Therefore, the measurement of estradiol in saliva is very attractive for clinicians due to its manifold advantages over venipuncture - being non-invasive, less stressful for the patient, multiple samples can be collected by a layperson after a short introduction.

Steroid hormones have distinct circadian rhythms, especially estradiol and progesterone in females are strongly correlated with the menstrual cycle [23; 24].

4. TEST PRINCIPLE

The 17beta-Estradiol Saliva ELISA is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding.
The microtiter wells are coated with a monoclonal antibody directed towards an antigenic site on the 17beta-Estradiol molecule. Endogenous 17beta-Estradiol of a patient sample competes with a Estradiol-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off.
The amount of bound peroxidase conjugate is inversely proportional to the concentration of 17beta-Estradiol in the sample. After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of 17beta-Estradiol in the patient sample.

5. WARNINGS AND PRECAUTIONS

1. For *in-vitro diagnostic* use only. For professional use only by GLP trained professionals.
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. Broken glass may cause injury. Handle glass vessels with caution.
5. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
6. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
7. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
8. Chemicals and prepared, used, unused or expired reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
9. The cleaning staff should be guided by the professionals regarding potential hazards and handling.
10. All serious incidents that have occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.
11. The device contains material of animal origin, which is certified apparently free of infectious or contagious diseases and injurious parasites. Still the material should be handled with extreme caution.

12. Avoid contact with Stop solution. It may cause skin irritations and burns.
13. 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.

6. STORAGE AND STABILITY

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

The unopened reagents are stable until the expiry date indicated. The kit is stable up to 6 months after the first opening (not exceeding the expiry date) when the Microtiterplate is packed in a tightly closed bag, the bottles are closed with their screw caps and the kit is stored at indicated storage temperature.

7. SPECIMEN COLLECTION AND STORAGE

Specimen collection

The patient should not eat, drink, chew gums or brush teeth for 30 minutes before sampling. Rinse mouth thoroughly with cold water 5 minutes prior to sample collection.
Do not collect samples when oral diseases, inflammation or lesions exist (blood contamination). Reddish colour is indicating blood contamination and leading to wrong results.
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 minutes at 2000 – 3000 x g to remove particulate material.
Due to the pulsatile dynamics of steroid secretion, repeated saliva sampling is recommended.
IBL recommends collecting 3 to 5 saliva samples within 2 hours. In the laboratory, equal volumes of the individual saliva samples can be mixed. This mixed sample results in a mean value, which represents the active hormone concentration in a reproducible way.

Sample Collection Device

Saliva can be collected in a suitable sampling device.

Specimen storage

Saliva samples can be stored at 2°C to 25°C for ≤ 7 days.
It is recommended to freeze samples and store at -20°C for long time storage (< 6 months).
It is recommended to limit the number of freeze /thaw cycles to a maximum of 3.
Keep away from heat or direct sunlight.

8. MATERIALS SUPPLIED

Quantity	Symbol	Origin	Component
1 x 12 x 8	MTP	BIO	**Microtiter Plate** Break apart strips. MTP (12 strips of 8 wells each) coated with donkey anti-sheep antibody and sheep anti-17beta-Estradiol antibody in solution containing bovine serum albumin. Vacuum dried.
1 x 6 x 1 mL	CAL A-F	BIO	**Standard A-F** Ready to use. 0; 2; 4; 8, 24; 64 pg/mL Contains 17beta-Estradiol in phosphate buffer, ≤ 0.5 % bovine serum albumin and ≤ 0.1% ProClin (w/w).
1 x 2 x 1 mL	CONTROL 1+2	BIO	**Control 1+2** Ready to use. Contains 17beta-Estradiol in phosphate buffer, ≤ 0.5 % bovine serum albumin and ≤ 0.1 % ProClin (w/w). Concentrations / acceptable ranges see QC certificate.
1 x 13 mL	ENZCONJ	BIO	**Enzyme Conjugate** Ready to use. Contains Estradiol conjugated to peroxidase, sheep anti-Estrone antibody, < 0.01 % ProClin 300 (w/w), ≤ 0.02 % Methylisothiazolinone and ≤ 0.02 % Bromonitrodioxane.
1 x 100 mL	WASHBUF CONC		**Wash Buffer Concentrate** (10x) Phosphate buffer containing 0.5 % Tween 20 (w/w).
1 x 15 mL	TMB SUBS		**TMB Substrat Solution** Ready to use. Contains 3,3',5,5' Tetramethylbenzidine solution.
1 x 15 mL	TMB STOP		**TMB Stop Solution** Ready to use. Contains: 1 M sulfuric acid
3 x	FOIL		**Adhesive Foil**

9. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volume: 50; 100; 250 µL
2. Vortex mixer
3. Sample Diluent (can be ordered separately from IBL under REF KLZZ731).
4. A suitable sampling device should be used.
5. 8-Channel Micropipette 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

10. 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 pre-treatment 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 perform duplicate measurements 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.

11. PRE-TEST SETUP INSTRUCTIONS

11.1. Preparation of concentrated components

Dilute / dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
100 mL	**WASHBUF CONC**	ad 1000 mL	bidist. water	1:10	Mix vigorously	2 - 8°C	4 weeks

11.2. Dilution of Samples

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

12. TEST PROCEDURE

1.	Pipette **50 µL** of each **Standard, Control and sample** into the respective wells of the Microtiter Plate.
2.	Thoroughly mix for 3 seconds.
3.	**Incubate 60 minutes** at **18 - 25°C** (Room temperature).
4.	Pipette **100 µL** of **Enzyme Conjugate** into each well.
5.	Thoroughly mix for 10 seconds.
6.	Cover plate with adhesive foil. **Incubate 60 minutes** at **18 - 25°C.**
7.	Remove adhesive foil. 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.
8.	Pipette **100 µL** of **TMB Substrate Solution** into each well.
9.	**Incubate 30 minutes** at **18 - 25°C** (manual). In case of automation of the assay the incubation time can be reduced to 25 minutes.
10.	Stop the substrate reaction by adding **100 µL** of **TMB Stop Solution** into each well. Briefly mix contents by gently shaking the plate. Colour changes from blue to yellow.
11.	**Measure** optical density with a photometer at **450 nm** (Reference-wavelength: 600-650 nm) within **15 minutes** after pipetting of the Stop Solution.

13. AUTOMATION

Automated protocols can be provided for open ELISA systems, e.g.: Freedom EVOlyzer®.
For further information please contact: ProductSupport.IBL@tecan.com
For the use of products on automated instruments it is absolutely necessary to perform and maintain a validation according to legal requirements. A successful validation of the process is a prerequisite for diagnostic use. The responsibility for the implementation and documentation of validation in accordance with the country-specific requirements is assumed by the organization or institution.

14. 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.

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.

It is recommended to participate at appropriate quality assessment trials.

15. CALCULATION OF RESULTS

The obtained ODs 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 could be omitted and the more plausible single value used).

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

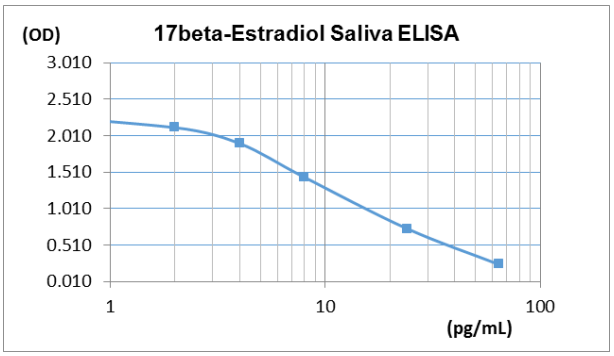
Samples showing concentrations above the highest standard have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and re-assayed.

Conversion: 17beta-Estradiol (pg/mL) x 3.67 = pmol/L

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	17beta-Estradiol	OD_{Mean}	OD/OD_{max}
A	0 pg/mL	2.433	100 %
B	2 pg/mL	2.122	87 %
C	4 pg/mL	1.904	78 %
D	8 pg/mL	1.441	59 %
E	24 pg/mL	0.735	30 %
F	64 pg/mL	0.245	10 %



Measuring Range: 2.1 pg/mL (LoQ as functional sensitivity) to 61.2 pg/mL.

16. EXPECTED VALUES

The results themselves should not be the only reason for any therapeutic 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.

The concentration of 17beta-Estradiol in women (17 - 80 years) was found to be between 3.0 - 11.9 pg/mL. Literature states a range between 0.54 - 4.78 pg/mL for postmenopausal women [5] and 0.98 - 11.91 pg/mL for premenopausal women [25; 24]. The range for girls during puberty is 0.82 - 4.3 pg/mL [25; 26].
The concentration of 17beta-Estradiol in men (24 and 63 years) was found to be between 2.1 - 4.1 pg/mL (median: 2.8 pg/mL); 10% - 90 % Percentile.
Literature states a range between 0.53 - 3.55 pg/mL for healthy men [25; 24].

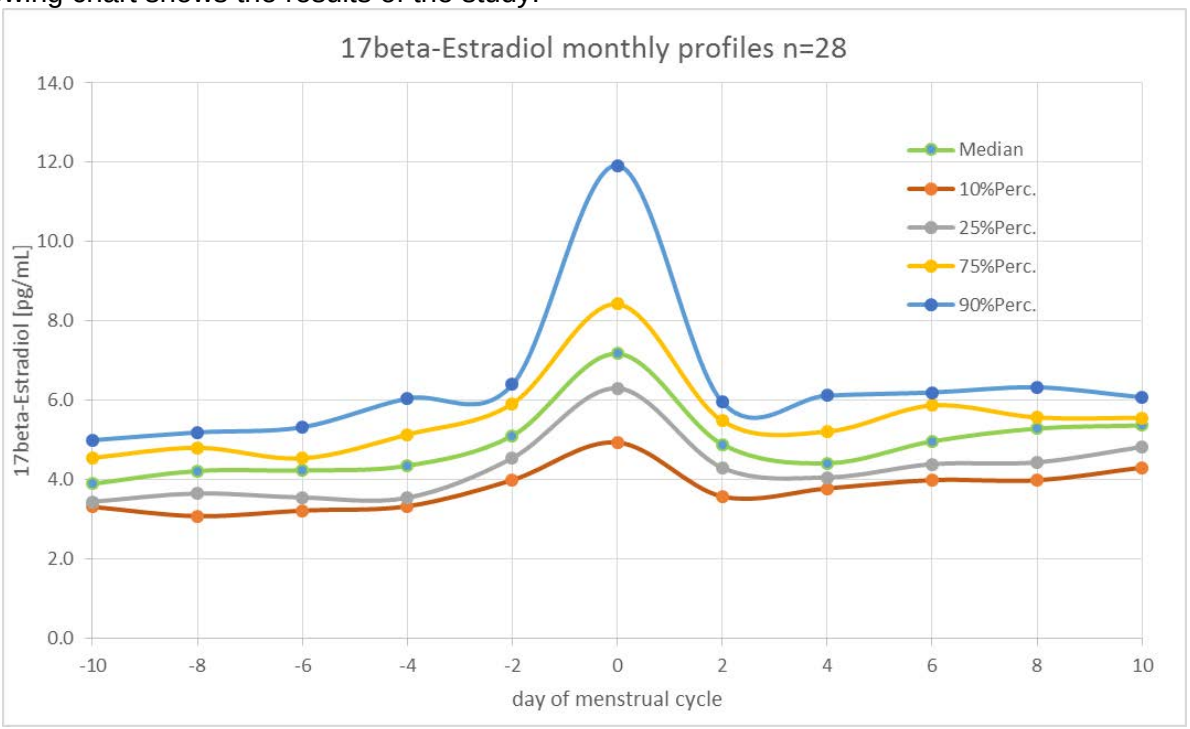
This is in accordance with the following data measured at IBL.

Apparently healthy subjects show the following values:

	n	Age	10% - 90% percentile		Median
Females (Premenopausal)	28	17 - 49 years	Follicular Phase:	3.1 - 6.4 pg/mL	4.4 pg/mL
			Midcycle:	4.9 - 11.9 pg/mL	7.2 pg/mL
			Luteal Phase:	3.6 - 6.3 pg/mL	5.0 pg/mL
Females (Postmenopausal)	117	65 - 80 years		3.0 - 7.5 pg/mL	4.2 pg/mL
Males	34	24 - 63 years		2.1 - 4.1 pg/mL	2.8 pg/mL

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 five times during a period of 2 hours after awakening covering the whole menstrual cycle with a maximum of 30 days. Samples were pooled per day and the estradiol concentration was measured to obtain a daily value throughout the menstrual cycle.

The following chart shows the results of the study:



Reference ranges in affected population

Literature states the following ranges for estrogen deficiency.

Population	Concentration	Method	Reference
Estrogen therapy (ET) Age: 48 - 65 years; n = 43 postmenopausal women (31 received estrogen, 12 none)	ET Mean: 1.61 ±0.23 pg/mL Controls: 1.39 ±0.33 pg/mL	Immunoassay	Tivis et al., 2005 [5]
Amenorrhea n = 4 women	5 - 18 pg/mL (proliferative phase) 8 - 35 pg/mL (secretory phase)	Immunoassay (RIA)	Choe et al., 1983 [27]

17. 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.

The following substances do not have a significant effect on the test results up to the below stated concentrations (+/- 20 %).

Substance	Concentration	Recovery of expected result
Citric acid	0.1 %	98 % (82 - 118 %)
Milk	1.0 %	99 % (91 - 111 %)
Blood	1.0 %	96 % (80 - 108 %)

Note: The 17beta-Estradiol Saliva ELISA should not be used for patients being treated with the drug Fulvestrant (Faslodex®) and/or with drugs containing Epiestriol, as those cross react in the 17beta-Estradiol Saliva ELISA and could lead to falsely elevated test results.

18. PERFORMANCE

18.1. Analytical Specificity (Cross Reactivity)

The cross reactivity study was conducted during eight days of testing by one operator. A maximum of one run were performed per day with high and low kit controls and with three different human saliva samples spiked with potential interfering substance in six different concentrations (0, 0.1, 1, 10, 100, 1000, 10000 and 100000 pg/mL). Each sample was tested in duplicate using one reagent lot.

Substance	Cross Reactivity
16-Epiestriol	26.6 %
Estradiol-3-Glucoronide	2.93 %
Fulvestrant	2.49 %
Medroxyprogesterone	1.57 %
Estrone	1.03 %
Estriol	0.73 %
Dihydrotestosterone	0.21 %
Ethinylestradiol	0.05 %
Dehydroepiandrosterone (DHEA)	0.04 %
Estrone-3-sulfate	0.03 %
alpha-Estradiol	0.02 %
11-Deoxycorticosterone	≤ 0.01 %
11-Deoxycortisol	≤ 0.01 %
17-alpha-Hydroxypregnenolone	≤ 0.01 %
17-alpha-Hydroxyprogesterone	≤ 0.01 %
17-alpha-Methyltestosterone	≤ 0.01 %
21-Deoxycortisol	≤ 0.01 %

Substance	Cross Reactivity
6-beta-Hydroxycortisol	≤ 0.01 %
Androstenedione	≤ 0.01 %
Androsterone	≤ 0.01 %
Corticosterone	≤ 0.01 %
Cortisol	≤ 0.01 %
Cortisone	≤ 0.01 %
Dexamethasone	≤ 0.01 %
Estriol-16-glucoronide	≤ 0.01 %
Medroxyprogesterone-17-acetate	≤ 0.01 %
Mifepristone	≤ 0.01 %
Norethisterone	≤ 0.01 %
Norgestrel	≤ 0.01 %
Prednisone	≤ 0.01 %
Prednisolone	≤ 0.01 %
Pregnenolone	≤ 0.01 %
Progesterone	≤ 0.01 %
Testosterone	≤ 0.01 %

Limit of Blank (LoB)
The LoB study was performed with the zero calibrator (Standard A), measured in duplicates per sample over three days with two kit lots. Limit of Blank = 1.3 pg/mL

Limit of Detection (LoD)
The LoD study was performed using four different low concentrated samples, measured in 3 replicates per sample over three days with two kit lots. Limit of Detection = 2.1 pg/mL

Limit of Quantitation (LoQ as functional sensitivity)
The LoQ as functional sensitivity study was performed using 7 different samples. 4 low concentrated samples were measured in 3 replicates and 3 higher concentrated samples were measured in 4 replicates per sample over three days with two kit lots. Limit of Quantitation as functional sensitivity = 2.1 pg/mL

Metrological Traceability
The concentrations assigned to kit calibrators and controls are traceable to reference measurement procedure LC-MS/MS through an unbroken chain of calibration according to EN ISO 17511:2021.
The combined uncertainty of measurement is calculated as 17.6 % for the determination of 17beta-Estradiol in saliva according to the 'Guide to the expression of uncertainty in measurement' (JCGM 100:2008).
The metrological traceability is validated by the comparison between the chosen reference procedure LC-MS/MS and IBL 17beta-Estradiol Saliva ELISA. The coefficient of correlation r = 0.975 indicates a good correlation between the reference procedure LC-MS/MS and IBL 17beta-Estradiol Saliva ELISA.

Method Comparison
Clinical evidence was shown by comparing the IBL 17beta-Estradiol Saliva ELISA to LC-MS/MS (gold standard) predetermined samples.
The method comparison study was conducted during one day of testing by one operator. The run was performed with high and low kit controls and with the different human saliva samples. Each sample was tested in duplicate using one reagent lot. The correlation between the IBL ELISA assay and LC-MS/MS was determined by Passing-Bablok regression. IBL-Assay = 1.0856 x LC-MS/MS – 0.7627; r = 0.98; n = 50

Linearity
The linearity study was performed measuring three different saliva samples with concentrations between 6.7 and 61.2 pg/mL in serial dilution. The assay showed linear behavior up to a 1:8 dilution.
The mean recovery in dilution was 93.9 % (Range: 86.1 - 103.5 %).

Recovery
The recovery study was performed measuring three different saliva samples with concentrations between 4.5 and 20.1 pg/mL. Increasing amounts of Estradiol were added to the saliva samples.
The mean recovery in dilution was 87.9 % (Range: 80.5 % - 96.1 %).

Precision
The intra assay study was conducted during 20 days using one reagent lot. Two runs were performed per day with high and low kit controls and with a panel of 4 human saliva samples. Each sample was run in duplicate.

Sample	Mean conc.	within run (Intra-Assay)	
		SD	CV
1	2.31 pg/mL	0.39 pg/mL	16.8 %
2	5.08 pg/mL	0.37 pg/mL	7.30 %
3	12.9 pg/mL	0.66 pg/mL	5.10 %
4	26.1 pg/mL	1.53 pg/mL	5.90 %

The intra assay precision calculated from four different human saliva samples showed a mean CV of 8.78 % (Range: 5.1 % - 16.8 %).

The inter assay study was conducted during 20 days using one reagent lot. Two runs were performed per day with high and low kit controls and with a panel of 4 human saliva samples. Each sample was run in duplicate.

Sample	Mean conc.	total precision (Inter-Assay)	
		SD	CV
1	2.31 pg/mL	0.51 pg/mL	22.3 %
2	5.08 pg/mL	0.54 pg/mL	10.7 %
3	12.9 pg/mL	0.89 pg/mL	6.90 %
4	26.1 pg/mL	1.92 pg/mL	7.40 %

The inter assay precision calculated from four different human saliva samples showed a mean CV of 11.8 % (Range: 6.9 % - 22.3 %).

The inter lot variation study was conducted during 5 days of testing. Each run was performed with high and low kit controls and with a panel of 4 human saliva samples. Each sample was tested in triplicate per run with 3 different reagent lots.

Sample	Mean conc.	total precision (Inter-Lot)	
		SD	CV
1	3.96 pg/mL	0.68 pg/mL	17.1 %
2	7.08 pg/mL	0.61 pg/mL	8.6 %
3	13.0 pg/mL	0.97 pg/mL	7.5 %
4	28.7 pg/mL	3.47 pg/mL	12.1 %

The inter lot variation calculated from four different human saliva samples showed a mean CV of 11.3 % in a range of 7.5 - 17.1 %.

Measuring Range
The measuring range is defined between the LoQ (as functional sensitivity) and the highest concentration that showed a linear behavior. The measuring range for the 17beta-Estradiol Saliva ELISA is between 2.1 pg/mL (LoQ as functional sensitivity) and 61.2 pg/mL.

19. PRODUCT LITERATURE REFERENCES

[1] Ryan, K. J. (1982). Biochemistry of aromatase: significance to female reproductive physiology. Cancer research, 42(8 Supplement), 3342s-3344s.

[2] Greenspan, F. S., & Gardner, D. G. (2001). Basic and clinical endocrinology.

[3] Gavrilova, N., & Lindau, S. T. (2009). Salivary sex hormone measurement in a national, population-based study of older adults. Journals of Gerontology Series B: Psychological Sciences and Social Sciences, 64(suppl_1), i94-i105.

[4] Mendoza, K., Curran, M., Salimetrics, L. L. C., & Lindau, S. T. (2007). Salivary Estradiol Measurement in Wave I of the Social Life Health & Aging Project. J Gerontol B Psychol Sci Soc Sci. 2009 Nov; 64B(Suppl 1): i94–i105. doi: 10.1093/geronb/gbn028

[5] Tivis, L. J., Richardson, M. D., Peddi, E., & Arjmandi, B. (2005). Saliva versus serum estradiol: implications for research studies using postmenopausal women. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 29(5), 727-732.

[6] Jameson JL, De Groot LJ (25 February 2015). Endocrinology: Adult and Pediatric E-Book. Elsevier Health Sciences. p. 2179. ISBN 978-0-323-32195-2.

[7] Shirtcliff, E. A., Dahl, R. E., & Pollak, S. D. (2009). Pubertal development: correspondence between hormonal and physical development. Child development, 80(2), 327-337.

[8] Fernández-Garcia, B., Lucia, A., Hoyos, J., Chicharro, J. L., Rodriguez-Alonso, M., Bandres, F., & Terrados, N. (2002). The response of sexual and stress hormones of male pro-cyclists during continuous intense competition. International journal of sports medicine, 23(08), 555-560.

[9] Ikegami, S., Moriwake, T., Tanaka, H., Inoue, M., Kubo, T., Suzuki, S., ... & Seino, Y. (2001). An ultrasensitive assay revealed age-related changes in serum oestradiol at low concentrations in both sexes from infancy to puberty. Clinical endocrinology, 55(6), 789-795.

[10] Kang, J. Y., Park, J. Y., Chun, S. I., Suh, H. S., Lee, K., & Ahn, R. S. (2014). Puberty-related changes in cortisol, dehydroepiandrosterone, and estradiol-17β secretions within the first hour after waking in premenarcheal girls. Neuroendocrinology, 99(3-4), 168-177.

[11] McIver, B., Romanski, S. A., & Nippoldt, T. B. (1997, December). Evaluation and management of amenorrhea. In Mayo Clinic Proceedings (Vol. 72, No. 12, pp. 1161-1169). Elsevier.

[12] Buckler, H. (2005). The menopause transition: endocrine changes and clinical symptoms. British Menopause Society Journal, 11(2), 61-65.

[13] Goletiani, N. V., Keith, D. R., & Gorsky, S. J. (2007). Progesterone: Review of safety for clinical studies. Experimental and clinical psychopharmacology, 15(5), 427.

[14] Bowen R (2000-08-06). "Placental Hormones". Retrieved 2020-29-12. http://www.vivo.colostate.edu/hbooks/pathphys/reprod/placenta/endocrine.html

[15] Hall, J. E. (2004). Neuroendocrine control of the menstrual cycle. In J. F. Strauss, III, & R. L. Barbieri (Eds.), Yen and Jaffe's reproductive endocrinology: Physiology, pathophysiology, and clinical management (5th ed., pp. 195–211). Philadelphia: Elsevier

[16] Cable, J. K., & Grider, M. H. (2020). Physiology, Progesterone. StatPearls

[17] Frank, G. R. (2003). Role of estrogen and androgen in pubertal skeletal physiology. Medical and pediatric oncology, 41(3), 217-221.

[18] Herrera et al., 2017: Herrera, A. Y., Hodis, H. N., Mack, W. J., & Mather, M. (2017). Estradiol therapy after menopause mitigates effects of stress on cortisol and working memory. The Journal of Clinical Endocrinology & Metabolism, 102(12), 4457-4466

[19] Vermeulen, A., Kaufman, J. M., Goemaere, S., & Van Pottelberg, I. (2002). Estradiol in elderly men. The aging male, 5(2), 98-102.

[20] Dielen, C., Fiers, T., Somers, S., Deschepper, E., & Gerris, J. (2017). Correlation between saliva and serum concentrations of estradiol in women undergoing ovarian hyperstimulation with gonadotropins for IVF/ICSI. Facts, views & vision in ObGyn, 9(2), 85.

[21] Lu, Y. C., Bentley, G. R., Gann, P. H., Hodges, K. R., & Chatterton, R. T. (1999). Salivary estradiol and progesterone levels in conception and nonconception cycles in women: evaluation of a new assay for salivary estradiol. Fertility and sterility, 71(5), 863-868.

[22] Bacon, J. L. (2017). The menopausal transition. Obstetrics and Gynecology Clinics, 44(2), 285-296.

[23] Gandara, B. K., Leresche, L., & Mancl, L. (2007). Patterns of salivary estradiol and progesterone across the menstrual cycle. Annals of the New York Academy of Sciences, 1098, 446.

[24] Schmidt, J., Wenzel, F., & Blessing, F. (2020) Practical evaluation of two commercial immunoassays for the quantification of steroid hormones in human saliva. Journal of Cellular Biotechnology, (Preprint), 1-9.

[25] Shirtcliff, E. A., D. A. Granger, et al. (2000). "Assessing estradiol in biobehavioral studies using saliva and blood spots: simple radioimmunoassay protocols, reliability, and comparative validity." Horm Behav 38(2): 137-47

[26] Quaiser-Pohl, C., Jansen, P., Lehmann, J., & Kudielka, B. M. (2016). Is there a relationship between the performance in a chronometric mental-rotations test and salivary testosterone and estradiol levels in children aged 9–14 years?. Developmental psychobiology, 58(1), 120-128.

[27] Choe, J. K., Khan-Dawood, F. S., & Yusoff-Dawood, M. (1983). Progesterone and estradiol in the saliva and plasma during the menstrual cycle. American journal of obstetrics and gynecology, 147(5), 557-562

Symbols / Symbole / Symboles / Símbolos / Simboli / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.–Cat.: / N.º Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lotto n.: / Lote N.º: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Da utilizzare entro:/ Usar até: / Χρησιμοποιείται από:
Σ	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / Quantità dei tests: / N.º de Testes: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrato / Concentrado / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizzato / Liofilizado / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device / In-vitro-Diagnostikum / Appareil Médical pour Diagnostics In Vitro / Dispositivo Médico para Diagnóstico In Vitro / Dispositivo Medico Diagnostico In vitro / Equipamento Médico de Diagnóstico In Vitro / Ιατρική συσκευή για In-Vitro Διάγνωση
BIO	Contains biological material of human origin / Enthält biologisches Material menschlichen Ursprungs / Contient une substance biologique d'origine humaine / Contiene material biológico de origen humano / Contiene materiale biologico di origine umana / Contém material biológico de origem humana / Περιέχει βιολογικό υλικό ανθρώπινης προέλευσης
BIO	Contains biological material of animal origin / Enthält biologisches Material tierischen Ursprungs / Contient une substance biologique d'origine animale / Contiene material biológico de origen animal / Contiene materiale biologico di origine animale / Contém material biológico de origem animal / Περιέχει βιολογικό υλικό ζωικής προέλευσης
UDI	Unique Device Identification / Eindeutige Gerätekennung / Identifiant de dispositif unique / Identificación única de producto / Identificatore univoco del dispositivo / Identificador de dispositivo único / Μοναδικός αναγνωριστικός κωδικός προϊόντος
	Read instructions before use / Arbeitsanleitung lesen / Lire la fiche technique avant emploi / Lea las instrucciones antes de usar / Leggere le istruzioni prima dell'uso / Ler as instruções antes de usar / 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 / Non esporre ai raggi solari / Manter longe do calor ou luz solar directa / 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: / Armazenar em: / Αποθήκευση στους:
2-8°C	Store at: 2 - 8°C / Lagern bei: 2 - 8°C / Stocker à: 2 - 8°C / Almacene a: 2 - 8°C / Armazenar a: 2 - 8°C / Conservare a: 2-8°C / Armazenar em: 2-8°C / Αποθήκευση στους: 2-8°C
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Distributor: / Distributor: / Distributeur: / Distributor: / Distributore: / Distribuidor: / Διανομέας:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Attenzione! / Cuidado! / 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. Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT. Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS. Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

Generic table, not all symbols are present in the product

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.

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