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Instructions for use

Estradiol Saliva ELISA Free

REF **SA E-6200**

2°C – 8°C

Σ 96

IVD

CE

Estradiol Saliva ELISA

English

1. INTRODUCTION

1.1 Intended Use

Enzyme immunoassay for the *in vitro diagnostic* quantitative measurement of active free Estradiol, an estrogenic steroid, in saliva.
Results may be used to assess fertility problems in women; to diagnose menopause, and to monitor hormone replacement therapy.

1.2 Summary and Explanation

Estradiol (1,3,5(10)-estratriene-3,17β-diol; 17β-estradiol; E21) is a C18 steroid hormone with a molecular weight of 272.4 Dalton. It is the most potent natural Estrogen, produced mainly by the Graafian follicle of the female ovary and the placenta, and in smaller amounts by the adrenals, and the male testes (1-3).
Estradiol (E2) is secreted into the blood stream where 98% of it circulates bound to sex hormone binding globulin (SHBG) and to a lesser extent to other serum proteins such as albumin. Only a small fraction circulates as free hormone or in the conjugated form (4,5). Estrogenic activity is effected via estradiol-receptor complexes which trigger the appropriate response at the nuclear level in the target sites. These sites include the follicles, uterus, breast, vagina, urethra, hypothalamus, pituitary and to a lesser extent the liver and skin.
In non-pregnant women with normal menstrual cycles, estradiol secretion follows a cyclic, biphasic pattern with the highest concentration found immediately prior to ovulation (6,7). The rising estradiol concentration is understood to exert a positive feedback influence at the level of the pituitary where it influences the secretion of the gonadotropins, follicle stimulating hormone (FSH), and luteinizing hormone (LH), which are essential for follicular maturation and ovulation, respectively (8). Following ovulation, estradiol levels fall rapidly until the luteal cells become active resulting in a secondary gentle rise and plateau of estradiol in the luteal phase. During pregnancy, maternal serum Estradiol levels increase considerably, to well above the pre-ovulatory peak levels and high levels are sustained throughout pregnancy (9).

Estradiol affects a diversity of biological processes. Estradiol is responsible for conceptus-endometrial interactions during pregnancy (10), and adds to define the reproductive age (11). Furthermore, Estradiol is involved in brain development, memory and psychopathology, and affects cognitive functions (12). Moreover, changes in immune response are related to use or cessation of hormone replacement at menopause. In postmenopausal women, estrogen deprivation has been attributed to changes of the immune system (13). Estradiol and androgens are responsible for subtle cardiovascular changes long before the development of overt atherosclerosis and contribute to the fact that females are at lower risk of developing cardiovascular disease (CVD) as compared to males (14). Finally, reproductive effects and later life salivary sex steroid hormone levels may have independent effects on later life breast density and cancer risk (15).
There are potential clinical and research applications of diagnostics based on oral fluids (16).However, correct saliva sampling, storage and sample preparation is important for reliable results, and circadian rhythm must be taken into account (17, 18).

2. PRINCIPLE OF THE TEST

The Estradiol Saliva ELISA kit is based on the competition principle and the microplate separation.
An unknown amount of Estradiol present in the sample and a fixed amount of Estradiol conjugated with horseradish peroxidase compete for the binding sites of a polyclonal Estradiol antiserum coated onto the wells.
After two hours incubation the microtiter plate is washed to stop the competition reaction. Having added the substrate solution the concentration of Estradiol is inversely proportional to the optical density.

3. WARNINGS AND PRECAUTIONS

1. This kit is for in vitro diagnostic use only. For professional use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of instructions for use provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C - 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.

6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21 °C - 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with Stop Solution containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from the manufacturer.

4. REAGENTS

4.1 Reagents provided

SA E-6231 96 **Microtiterwells**

Contents: 12 x8 (break apart) strips, 96 well;
Wells coated with anti-Estradiol antibody (polyclonal).

Standards and Controls- ready to use

Cat. no	Component	Concentration [pg/ml]	Volume / Vial
SA E-6201	STANDARD A	0	1 ml
SA E-6202	STANDARD B	1	1 ml
SA E-6203	STANDARD C	5	1 ml
SA E-6204	STANDARD D	10	1 ml
SA E-6205	STANDARD E	50	1 ml
SA E-6206	STANDARD F	100	1 ml
SA E-6251	CONTROL 1	Control values and ranges please refer to vial label or QC-Report.	1 ml
SA E-6252	CONTROL 2		1 ml

Contents: Contain non-mercury preservative.

SA E-6240 CONJUGATE **Enzyme Conjugate** - Ready to use

Contents: Estradiol conjugated to horseradish peroxidase;
Contain non-mercury preservative.

Volume: 1 x 14 ml

FR E-0055 SUBSTRATE **Substrate Solution** - Ready to use

Contents: Tetramethylbenzidine (TMB).

Volume: 1 x 14 ml

FR E-0080 STOP-SOLN **Stop Solution** - Ready to use

Contents: 0.5 M H_2SO_4
Avoid contact with the stop solution. It may cause skin irritations and burns.

Volume: 1 x 14 ml

Hazards identification: 

H290 May be corrosive to metals.
H314 Causes severe skin burns and eye damage.

FR E-0030 WASH- CONC 40x **Wash Solution** - concentrated

Volume: 1 x 30 ml
Concentrate for 1200 ml.

Note: *Sample Diluent* for sample dilution is available upon request.

4.2 Material required but not provided

- Calibrated EIA reader adjusted to read at 450 nm
- Calibrated variable precision micropipettes (100 µl and 200 µl)
- Distilled or Deionized water
- Timer (60 min range)
- Reservoirs (disposable)
- Test tube or microtube rack in a microplate configuration
- Semi-logarithmic graph paper or software for data reduction.

4.3 Storage Conditions

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for two months if stored as described above.

4.4 Reagent Preparation

Bring all reagents to room temperature before use.

Wash Solution
Add deionized water to the 40X concentrated Wash Solution.
Dilute 30 ml of concentrated *Wash Solution* with 1170 ml deionized water to a final volume of 1200 ml.
The diluted Wash Solution is stable for 2 weeks at room temperature.

4.5 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Safety Data Sheet.

4.6 Damaged Test Kits

In case of any severe damage to the test kit or components, the manufacturer has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5. SPECIMEN COLLECTION AND PREPARATION

Eating, drinking, chewing gums or brushing teeth should be avoided for 30 minutes before sampling. Otherwise, it is recommended to rinse mouth thoroughly with cold water 5 minutes prior to sampling.

Do not collect samples when oral diseases, inflammation or lesions exist (blood contamination).
In case of visible blood contamination the patient should discard the sample, rinse the sampling device with water, wait for 10 minutes and take a new sample.

Note: Samples containing sodium azide should not be used in the assay.

5.1 Specimen Collection

It is recommended to collect saliva samples with commercially available equipment (e.g. Sali Set, catalogue no. SA D-6100, 100 pieces).

Do not use any cotton swab for sampling, such as Salivettes; this in most cases will result in significant interferences. Due to the episodic secretion pattern of steroid hormones it is important to care for a proper timing of the sampling.

In order to avoid arbitrary results we recommend that always 5 samples be taken within a period of 2 - 3 hours (*multiple sampling*) preferably before a meal.
As food might contain significant amounts of steroid hormones samples preferably should be taken while fasting. If fasting should be a problem the collection period should be timed just before lunch or before dinner.

5.2 Specimen Storage and Preparation

Specimens should be capped and may be stored for up to one week at 4 °C prior to assaying.
Specimens held for a longer time should be frozen -20 °C prior to assay. Even repeated thawing and freezing is no problem.

Each sample has to be frozen, thawed, and centrifuged at least once anyhow in order to separate the mucins by centrifugation.
Upon arrival of the samples in the lab the samples have to stay in the deep freeze at least overnight. Next morning the frozen samples are warmed up to room temperature and mixed carefully.
Then the samples have to be centrifuged for 5 to 10 minutes (at 3000 - 2000 × *g*).
Now the clear colorless supernatant is easy to pipette.

If a set of multiple samples have to be tested, the lab (after at least one freezing, thawing, and centrifugation cycle) has to mix the aliquots of the 5 single samples in a separate sampling device and perform the testing from this mixture.

5.3 Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Sample Diluent* and re-assayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.

Example:
a) Dilution 1:10: 10 µl saliva + 90 µl *Sample Diluent* (mix thoroughly)
b) Dilution 1:100: 10 µl of dilution a + 90 µl *Sample Diluent* (mix thoroughly).

6. ASSAY PROCEDURE

6.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

6.2 Test Procedure

Each run must include a standard curve.
All standards, samples, and controls should be run in duplicate. All standards, samples, and controls should be run concurrently so that all conditions of testing are the same.

1.	Secure the desired number of Microtiter wells in the holder.
2.	Dispense **100 µl** of each ***Standard, Control*** and samples with new disposable tips into appropriate wells.
3.	Incubate for **60 minutes** at room temperature.
4.	Dispense **100 µl** of ***Enzyme Conjugate*** into each sample and standard well Mix the plate thoroughly for 10 seconds. It is important to have a complete mixing in this step.
5.	Incubate for **60 minutes** at room temperature.
6.	Briskly shake out the contents of the wells. Rinse the wells **3 times** with diluted Wash Solution (400 µl per well). Strike the wells sharply on absorbent paper to remove residual droplets. **Important note:** The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
7.	Add **100 µl** of ***Substrate Solution*** to each well.
8.	Incubate for **30 minutes** at room temperature.
9.	Stop the enzymatic reaction by adding **100 µl** of ***Stop Solution*** to each well.
10.	Determine the absorbance (OD) of each well at **450±10 nm** with a microtiter plate reader. It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

6.3 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using semi-logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 100 pg/ml. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3.1 Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard		Absorbance Units
Standard A	(0 pg/ml)	2.15
Standard B	(1 pg/ml)	1.88
Standard C	(5 pg/ml)	1.56
Standard D	(10 pg/ml)	1.33
Standard E	(50 pg/ml)	0.61
Standard F	(100 pg/ml)	0.30

7. EXPECTED NORMAL VALUES

In a study conducted with apparently normal healthy donors, using the Estradiol Saliva ELISA, the following values are observed:

Population		n	Mean [pg/ml]	Median [pg/ml]	2.5^{th} – 97.5^{th} Percentile [pg/ml]	Range (min. – max.) [pg/ml]
Males	16 - 57 years	54	1.04	0.93	0.16 – 2.41	0.13 – 3.36
Females	luteal phase	49	1.59	1.24	0.25 - 4.54	0.20 - 5.17
	follicular phase	21	1.52	1.05	0.28 - 3.72	0.28 - 3.75
	post-menopausal	12	0.66	0.42	0.07 - 2.49	0.06 - 3.07

Therapy should not be decided based on results alone. The results should be correlated to other clinical observations and diagnostic tests.
Salivary Estradiol values show a clear circadian rhythm. We therefore recommend collection of the saliva samples on the same hour each day.
Furthermore, we recommend that each laboratory establish its own range for the population tested, because the values differ between age, new born, children, adolescents and adults.

8. QUALITY CONTROL

Good laboratory practice requires that controls be run with each standard curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the QC-Laboratory are stated in the QC Report added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor or the manufacturer directly.

9. PERFORMANCE CHARACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between 0.04 – 100 pg/ml.

9.2 Specificity

The following materials have been evaluated for cross reactivity. The percentage indicates cross reactivity at 50% displacement compared to Estradiol.

Compound	% Cross reactivity
Estradiol-17ß	100
Estrone	1.77
Estriol	0.61
7-a-Ethinylestradiol	0.5
Androstenedione	0.03
Estradiol-17α	0.03
Corticosterone	0
17α-Hydroxyprogesterone	0
Dehydroepiandrosterone	0
Dihydrotestosterone	0
Drospirenone	0
Progesterone	0
Testosterone	0

9.3 Sensitivity

The sensitivity study was designed according to CLSI guideline EP17-A2.

Limit of Blank (LoB)	0.04 pg/ml
Limit of Detection (LoD)	0.21 pg/ml
Limit of Quantification (LoQ)	0.55 pg/ml

9.4 Reproducibility

9.4.1 Intra Assay

The intra-assay variation was determined by 10 replicate measurements of 4 saliva samples using *Estradiol Saliva ELISA* kit.

The within assay variability is shown below:

Sample	n	Mean [pg/ml]	CV (%)
1	10	8.12	7.01
2	10	26.45	6.31
3	10	32.17	6.79
4	10	92.54	1.42

9.4.2 Inter Assay

The inter-assay variation (between run) is determined with 4 samples. The 4 samples are measured in 3 days with 10 replicates per run. The between assay variability is shown below:

Sample	n	Mean [pg/ml]	CV (%)
1	30	8.08	5.67
2	30	29.48	9.38
3	30	34.96	13.40
4	30	93.20	1.65

9.5 Recovery

Recovery of the *Estradiol Saliva ELISA* was determined by adding increasing amounts of the analyte to 4 different saliva samples containing different amounts of endogenous analyte. Each sample (non-spiked and spiked) was assayed and analyte concentrations of the samples were calculated from the standard curve. The percentage recoveries were determined by comparing expected and measured values of the samples.

	Sample 1	Sample 2	Sample 3	Sample 4
Concentration [pg/ml]	44.03	64.4	74.84	76.5
Average % recovery	96.9	95.2	92.0	96.6
Range of Recovery % from	88.8	92.7	90.0	95.6
Range of Recovery % to	105.3	97.4	94.1	98.9

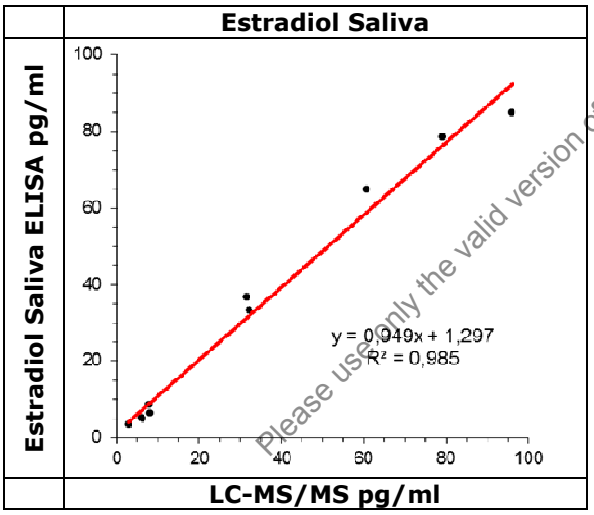
9.6 Linearity

4 samples (saliva) containing different amounts of analyte were serially diluted up to 1:16 with Sample Diluent and assayed with the *Estradiol Saliva ELISA*. The percentage recovery was calculated by comparing the expected and measured values for Estradiol Saliva.

	Sample 1	Sample 2	Sample 3	Sample 4
Concentration [pg/ml]	59.8	66.1	67.3	85.6
Average% recovery	103.9	98.1	95.5	102.2
Range of Recovery % from	98.1	94.9	89.4	87.4
Range of Recovery % to	106.7	106.4	100.8	113.3

9.7 Comparison Studies

A study was performed that evaluated 10 saliva samples collected from adult men and women. The samples were analyzed with the *Estradiol Saliva ELISA* and a LC-MS/MS to determine the concentration of estradiol in the saliva samples. A correlation coefficient of r = 0.992 and regression formula of y = 0.949 x – 1.297 were obtained.



10. LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.
Any improper handling of samples or modification of this test might influence the results.

The patient should not eat, drink, chew gum or brush teeth for 30 minutes 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).

10.1 Interfering Substances

Blood contamination of more than 0.16 % in saliva samples will affect results, and usually can be seen by eye. Therefore, samples containing any visible blood should not be used.

Concentrations of Sodium Azide ≥ 0.02% interferes in this assay and may lead to false results.

10.2 Drug Interferences

The *Estradiol Saliva ELISA* should not be used for patients being treated with the drug fulvestrant (Faslodex®) which cross reacts in the *Estradiol Saliva ELISA* and could lead to falsely elevated test results.

10.3 High-Dose-Hook Effect

No hook effect was observed in this test.

11. LEGAL ASPECTS

Only for countries where the declaration of European Conformity (CE mark) is applicable.

11.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact the manufacturer.

11.2 Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient. Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

11.3 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2 are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

12. REFERENCES / LITERATURE

1. Tsang B.K., et al. (1980) Steroid biosyntheses by isolated human ovarian follicular cells in vitro, J. Clin. Endocrinol. Metab. 51, 1407 – 11
2. Gore-Langton R. E. et al. (1988): Follicular steroidogenesis and its control. In: The physiology of Reproduction. ED.: Knobil et al. pp.331-385, Raven press, New York
3. Hall, P.F. (1988): Steroid synthesis: Organization and Regulation. In: The Physiology of Reproduction. Ed.: Knobil et al., pp 975 – 988, Raven press, New York
4. Siiteri P.K. et al. (1982): The serum transport of steroid hormones. Rec. Progr. Horm. Res. 38, 457 – 510
5. Martin B. et al. (1981): Binding of steroids by proteins in follicular fluid of the human ovary. J. Clin. Endocrinol. Metab. 35, 443 – 447
6. Baird D.T. (1976): Ovarian steroid secretion and metabolism in women. In: The Endocrine function of the human ovary. Eds.: James V.H.T., Serio M. and Guisti G., pp. 125 – 33, Academic press, New York
7. McNtty K.P. et al. (1976): Concentration of estrogens and androgens in human ovarian venous plasma and follicular fluid throughout the menstrual cycle. J. Endocrinol. 71, 77 –85
8. March C.M. et al. (1979): Roles of estradiol and progesterone in eliciting mid-cycle luteinizing hormone and follicle stimulating hormone surges. J. Clin. Endocrinol. Metab. 49, 507 – 12.
9. Simpson E.R. and McDonald P.C. (1981): Endocrinology of pregnancy. In: Textbook of Endocrinology, Ed.: Williams R.H. pp. 412 – 22, Saunders Company, Philadelphia
10. Bazer F.W., Wu, G., Spencer, T.E., Johnson, G.A., Burghardt, R.C., & Bayless, K. (2009). Novel pathways for implantation and establishment and maintenance of pregnancy in mammals. Mol Hum Reprod, 16(3), 135-52.
11. Scheffer G.J., et al. (2003). The number of antral follicles in normal women with proven fertility is the best reflection of reproductive age. Hum Reprod, 18(4), 700-6.
12. McEwen, B.S. (1999). The molecular and neuroanatomical basis for estrogen effects in the central nervous system. J Clin Endocrinol Metab, 84(6), 1790-97.
13. Gameiro C.M., Romão F., and Castelo-Branco C. (2010) Menopause and aging: changes in the immune system--a review. Maturitas, 67(4), 316-20.
14. Perez-Lopez, F.R. et al. (2010). Gender differences in cardiovascular disease: Hormonal and biochemical influences. Reprod Sci, 17(6), 511-31.
15. Mockus M. , et al. (2015). First pregnancy characteristics, postmenopausal breast density, and salivary sex hormone levels in a population at high risk for breast cancer. BBA Clinical, 3, 189–195
16. Lawrence H.. (2002).Salivary markers of systemic disease: noninvasive diagnosis of disease and monitoring of general health. J Can Dent Assoc. 68(3), 170-4.
17. Toone R.J. et al. (2013) Measurement of steroid hormones in saliva: Effects of sample storage condition. Scand J. Clin. Lab., Invest. 73(8): 615-21.
18. Shirtcliff E.A. et al. (2001). Use of salivary biomarkers in biobehavioral research: Cotton-based sample collection methods can interfere with salivary immunoassay results. Psychoneuroendocrinology, 26(2), 165-73

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	I V D	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number		