LDN®
IMMUNOASSAYS AND SERVICES
BIOGENIC AMINES & NEUROSCIENCE | ENDOCRINOLOGY | FOOD SAFETY
LABOR DIAGNOSTIKA NORD GmbH & Co.KG | Am Eichenhain 1 | 48531 Nordhorn | Germany | Tel. +49 5921 8197-0 | Fax +49 5921 8197-222 | info@ldn.de | www.ldn.de

Instructions for use

Estradiol Saliva ELISA Free

REF SA E-6200

2°C–8°C Σ 96 IVD CE

Estradiol Saliva ELISA Free

1. INTENDED USE

The **Estradiol Saliva ELISA Free** is a manual enzyme immunoassay for the **quantitative** measurement of active free Estradiol, an estrogenic steroid, in human saliva.
For *in vitro* diagnostic use. For laboratory professional use.

The device is **intended to be used** to assess fertility problems in women; to diagnose menopause, and to monitor hormone replacement therapy.
The device is **not intended** to be used as a biomarker for the diagnosis of the breast cancer.

1.1 Scientific Validity Report

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 granulosa cells of the female ovary and the placenta by the aromatization of androstenedione to estrone, followed by conversion of estrone to estradiol by 17β HSD. Estradiol is also synthesized in other tissues including testicles, adrenal gland, fat tissue, liver, breast, and brain (1 5).
Estradiol promotes cell proliferation and inhibits apoptosis in tumors (6 – 8). For this reason, estradiol plays important roles in the development of breast cancer especially in premenopausal and postmenopausal women (9 – 10). The plasma and saliva estradiol concentrations are significantly increased in breast cancer patients compared to those in healthy women (11 – 12).
In plasma, estradiol is largely bound to SHBG and albumin. Only a fraction of 2.21% is free and biologically active, the percentage remaining constant throughout the menstrual cycle (13 – 15). Estradiol acts primarily as an agonist of the estrogen receptor (ER) subtypes ERα and ERβ, nuclear steroid hormone receptors which trigger the appropriate response at the nuclear level in the target sites. These sites include follicles, uterus, breast, vagina, urethra, hypothalamus, pituitary and to a lesser extent liver and skin.
In the female, estradiol acts as a growth hormone for tissue of the reproductive organs. During the menstrual cycle, estradiol secretion follows a cyclic, biphasic pattern with the highest concentration found immediately prior to ovulation. This estradiol peak stimulates the hypothalamic-pituitary axis to secrete the gonadotropins follicle stimulating hormone (FSH) and luteinizing hormone (LH), which are essential for follicular maturation and ovulation. In the luteal phase, estradiol, in conjunction with progesterone, prepares the endometrium for implantation (16 – 22). During pregnancy, estradiol concentration increases due to placental production and high levels are sustained throughout pregnancy (23).
Salivary steroid levels can reflect the circulating level of free steroid rather than total levels in serum, which is explained by the absence of circulating estradiol binding proteins. Therefore, saliva provides an excellent specimen for monitoring estradiol levels across the menstrual cycle or during hormone replacement therapy (24 – 26).

2. PRINCIPLE OF THE TEST

The Estradiol Saliva ELISA Free is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the **principle of competitive binding**.
The microtiter wells are coated with a polyclonal antibody (rabbit) directed towards antigenic sites of the estradiol molecule.
During incubation, estradiol in the added sample competes with the added enzyme conjugate, which is estradiol conjugated to horseradish peroxidase, for binding to the coated antibody.
After a washing step to remove all unbound substances, the solid phase is incubated with the substrate solution. The colorimetric reaction is stopped by addition of stop solution, and optical density (OD) of the resulting yellow product is measured. The intensity of color is inversely proportional to the concentration of the analyte in the sample.
A standard curve is constructed by plotting OD values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

3. WARNINGS AND PRECAUTIONS

- This kit is for *in vitro* diagnostic use only. For laboratory professional use only.
- Before starting the assay, read the instructions for use completely and carefully. Use the valid version of instructions for use provided with the kit. Be sure that everything is understood.
- Do not mix or use components from kits with different lot numbers. It is advised not to interchange 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.
- Do not use reagents beyond expiry date as shown on the kit labels.
- Do not reuse microtiter wells.
- Reagents of other manufacturers must not be used together with the reagents of this test kit.
- All reagents in this kit are clear liquids, substrate solution is clear and colorless. Changes in its appearance may affect the performance of the test. In that case, contact the manufacturer.

- Microbial contamination of reagents or samples may give false results.
- Allow the reagents to reach room temperature (20 °C to 26 °C) before starting the test. Temperature will affect the optical density readings of the assay.
- All indicated volumes must be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
- 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 coloured. Do not pour reagents back into original vials as reagent contamination may occur.

General precautions

- Follow laboratory quality assurance and laboratory safety guidelines.
- Never pipet by mouth and avoid contact of reagents and samples with skin and mucous membranes.
- Do not smoke, eat, drink, or apply cosmetics in areas where samples or kit reagents are handled.
- Wear lab coats and disposable latex gloves when handling samples and reagents and where necessary safety glasses.

Biohazard information

- 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. However, no known test method can offer total assurance that no infectious agent is present.
- The device contains material of animal origin, which is certified apparently free of infectious or contagious diseases and injurious parasites.
- Bovine components originate from countries where BSE (Bovine spongiform encephalopathy) has not been reported.
- All materials and samples of human or animal origin must be handled as if capable of transmitting infectious diseases.
- Handling must be done in accordance with the procedures defined by appropriate national biohazard and safety guideline or regulation. Waste must be discarded according to local rules and regulations.

Information to chemical hazards and hazard classification

- Some reagents contain preservatives in non-declarable concentrations. Nevertheless, in case of contact with eyes or skin, flush immediately with water.
- Substrate Solution contains an ingredient in non-declarable concentrations which causes serious eye irritation. In case of possible contact with eyes, rinse immediately carefully and thoroughly with eye wash or water. After contact with skin, wash with plenty of water. Take-off contaminated clothing and wash it before reuse.
- Avoid contact with Stop Solution containing < 5% H_2SO_4. It may cause skin irritation and burns.
- Chemicals and prepared or used reagents must be treated as hazardous waste according to the national safety guideline or regulation.
- This product does not contain substances which have carcinogenic, mutagenic or toxic for reproduction (CMR) properties.

All reagents of this test kit do NOT contain hazardous substances in concentrations to be declared, a classification and labelling is not required.

For detailed information, please refer to the Safety Data Sheet, which is available upon request directly from the manufacturer.

4. MATERIALS

4.1 Materials provided with the kit

SA E-6231 96 **Microtiter plate** – Ready to use

Contents: 12 x 8 wells (break apart);
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	For control values and ranges please refer to vial label or QC-Datasheet.	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; Colored red.
Contains non-mercury preservative.

Volume: 1 x 26 ml

SA E-0055 SUBSTRATE **Substrate Solution** – Ready to use

Contents: Contains 3,3`,5,5`-tetramethylbenzidine (TMB).
Keep away from direct sun light.

Volume: 1 x 25 ml

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

Contents: Contains < 5% 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** – 40X concentrate

Volume: 1 x 30 ml
See "Reagent Preparation".

1 x Instructions for Use

1 x Certificate of Analysis (CoA)

4.2 Materials required but not provided

- A calibrated microtiter plate reader (450 nm, with reference wavelength at 620 nm to 630 nm)
- Calibrated variable precision micropipettes
- Manual or automatic equipment for rinsing microtiter plate wells
- Absorbent paper
- Distilled water
- 0.9% NaCl
- Timer
- Graph paper or software for data reduction

4.3 Storage and Stability of the Kit

Unopened kits and reagents as well as **opened reagents** must be stored at 2 °C to 8 °C.

The microplate must always be stored in the resealable aluminum pouch containing a desiccant. Do not open the pouch until it has reached room temperature. The microtiter plate consists of 12 individual strips. Each strip can be divided into 8 individual wells.
Unused wells must be immediately returned to the aluminum pouch with the desiccant and stored again tightly resealed at 2 °C to 8 °C.

Once opened, reagent vials must be closed tightly again.

	Storage Temperature	**Stability**
Unopened kits and unopened reagents	2 °C to 8 °C	Until the expiration date printed on the label. Do not use reagents beyond this date!
Opened kit	2 °C to 8 °C	8 weeks

4.4 Reagent Preparation

Bring all reagents and required number of strips to room temperature (20 °C to 26 °C) prior to use.

Wash Solution
Add distilled water to the 40X concentrated Wash Solution.
Dilute 30 ml of concentrated *Wash Solution* with 1170 ml distilled water to a final volume of 1200 ml.

Stability after dilution:	at 20 °C to 26 °C	1 week

4.5 Disposal of the Kit

The disposal of the kit and all used materials/reagents must be performed according to the national regulations. Special information for this product is given in the Safety Data Sheet, section 13.

4.6 Damaged Test Kits

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

5. SAMPLE COLLECTION, STORAGE AND PREPARATION

Human saliva can be used in this assay.
The sampling must be done in the morning **before** food intake, drinking or smoking. It is recommended to rinse mouth thoroughly with cold water 5 minutes prior to sampling.
If sampling must be done during the day, eating, drinking, smoking, chewing gums or brushing teeth should be avoided at least 2 hours before sampling.
Do not collect samples when oral diseases, inflammation or lesions exist (blood contamination).
Note: Samples containing sodium azide should not be used in the assay.

Please refer to chapters "Interfering Substances" and "Drug Interferences".

5.1 Sample Collection

Saliva samples should be collected using SALI-TUBES 100 (available upon request).
Do not use any cotton swab for sampling, such as Salivette® tubes; this in most cases will result in significant interferences.
Other saliva sampling devices have not been tested and should be validated under the responsibility of the user.
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 Sample Storage and Preparation

Fresh saliva samples
Immediately after arrival in the lab, fresh saliva samples should be **frozen at least overnight at -20 °C**.
Each saliva sample has to be frozen, thawed, and centrifuged in order to separate the mucins by centrifugation.
Storage: immediately at -20 °C

Then samples must be thawed and centrifuged for 5 to 10 minutes at 10 000 *g*.
Thereafter, the clear supernatant must be transferred into a fresh tube.
Only this clear supernatant can be used as sample for the ELISA.

If a set of multiple samples has to be tested, the lab has to **mix aliquots of the supernatant** of the 5 single samples in a separate sampling device and perform the testing from this mixture.

Supernatant

Stability	at 2 °C to 8 °C	5 days
	at -20 °C (in aliquots)	at least 24 months

The supernatant should be frozen only once.
Thawed supernatant should be inverted several times prior to testing!

6. ASSAY PROCEDURE

6.1 Procedural Notes

- All reagents and samples must be allowed to come to room temperature (20 °C to 26 °C) before use.
- All reagents must be mixed without foaming.
- Do not interchange caps of reagent vials to avoid cross-contamination.
- Use new disposal plastic pipette tips for each standard, control, or sample in order to avoid carry-over.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
- Mix the contents of the microtiter plate wells thoroughly to ensure good test results.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Once the test has been started, all steps must be completed without interruption and in the same sequence for each step.
- The enzymatic reaction is linearly proportional to time and temperature.
- Optical density is a function of the incubation time and temperature. Respect the incubations times and temperatures as given in chapter "Test Procedure".
- 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.
- **Important note to wash procedure:**
  Washing is critical. Improperly washed wells will give erroneous results. The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
- **Test performance using fully automated analysis devices:**
  Automated test performance using fully automated, open-system analysis devices is possible. However, the combination must be validated by the user.

6.2 Test Procedure

Each run must include a standard curve.
The controls serve as internal controls for the reliability of the test procedure. They must be assayed with each test run.
All standards, samples, and controls must be run in duplicate. All standards, samples, and controls must be run concurrently so that all conditions of testing are the same.
The given test procedure describes manual processing.
Important note: The accuracy of this assay is markedly influenced by the correct incubation temperature.

1.	Secure the desired number of microtiter wells in the frame holder.
2.	Pipette **100 µl** of each ***Standard, Control,*** and **sample** with new disposable tips into appropriate wells.
3.	Incubate for **30 minutes** at room temperature.
4.	Add **200 µl *Enzyme Conjugate*** into each well. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
5.	Incubate for **120 minutes** at room temperature.
6.	Wash the wells as follows: If the wash step is performed manually: Briskly shake out the contents of the wells. Rinse the wells **3 times** with **300 µl** diluted *Wash Solution* per well. If an automated plate washer is used: Rinse the wells **3 times** with **400 µl** diluted *Wash Solution* per well. At the end of the washing step, always strike the wells sharply on absorbent paper to remove residual droplets!
7.	Pipette **200 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.	Measure the optical density (OD) of the solution in each well at **450 nm (reading) and at 620 nm to 630 nm (background subtraction, recommended)** 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. The concentration of the samples can be read directly from the standard curve.
2. For duplicate determinations, the mean of the two optical density (OD) values for each standard, control, and patient sample must be taken. If the two values deviate substantially from one another, the manufacturer recommends retesting the samples.
3. Samples with concentrations exceeding the highest standard can be further diluted with 0.9% NaCl and re-assayed as described in "Test Procedure", or must be reported as > 100 pg/ml. For the calculation of the concentrations, this dilution factor must be considered.
   (*Example*: dilution 1:10: 20 µl supernatant + 180 µl 0.9% NaCl)
4. Automated method:
   The results in the instructions for use have been calculated automatically using a four-parameter logistic (4PL) curve fit. (4PL Rodbard or 4PL Marquardt are the preferred methods.) Other data reduction functions may give slightly different results.
5. Manual method:

   Using semi-logarithmic graph paper, construct a standard curve by plotting the (mean) OD obtained from each standard against its concentration with OD value on the vertical (Y) axis and concentration on the horizontal (X) axis.

   Determine the corresponding sample concentration from the standard curve by using the (mean) OD value for each sample.

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	Optical Density (450 nm)
Standard A (0 pg/ml)	1.89
Standard B (1 pg/ml)	1.71
Standard C (5 pg/ml)	1.48
Standard D (10 pg/ml)	1.20
Standard E (50 pg/ml)	0.46
Standard F (100 pg/ml)	0.32

7. REFERENCE VALUES

It is strongly recommended that each laboratory determine its own reference values.

In a study conducted with apparently healthy adults, using the Estradiol Saliva ELISA ^{Free} the following data were observed:

Population	n	Mean (pg/ml)	Median (pg/ml)	2.5th – 97.5th Percentile (pg/ml)	Range (min. – max.) (pg/ml)
Males	28	1.11	1.00	0.72 – 3.30	0.72 – 4.78
Females					
Pre-menopausal					
follicular phase	10	3.78	3.64	1.90 – 6.90	1.78 – 6.99
luteal phase	10	2.47	2.63	1.20 – 3.60	1.05 – 3.75
Post-menopausal	22	2.12	2.19	0.72 – 4.4	0.72 – 4.56
Stages of pregnancy					
1.Trimester	10	4.48	4.29	2.26 – 6.83	1.97 – 6.96
2.Trimester	10	15.17	15.32	3.43 – 28.13	2.54 – 28.81
3.Trimester	10	101.64	108.90	35.05 – 172.23	31.87 – 178.47

The results alone should not be the only reason for any therapeutic consequences. The results must be correlated to other clinical observations and diagnostic tests.
Salivary Estradiol values show a clear circadian rhythm. We therefore recommend the saliva samples be obtained 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, newborn, children, adolescents and adults.

8. QUALITY CONTROL

Good quality assurance in the laboratory requires that controls be run with each calibration 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 Quality Control Laboratory are stated in the Certificate of Analyses (CoA) added to the kit. The values and ranges stated on the CoA always refer to the current kit lot and must be used for direct comparison of the results.
If available, it is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Apply appropriate statistical methods for analyzing control values and trends. If the results of the assay do not agree with 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 Specificity of Antibodies (Cross-Reactivity)

The following substances were tested for cross-reactivity of the assay:

Compound	% Cross reactivity
Estradiol-17β	100
Androstenedione	0.00
Androsterone	0.00
Corticosterone	0.00
Cortisone	0.00
Epiandrosterone	0.00
16-Epiestriol	0.00
Estradiol-3-sulfate	0.00
Estradiol-3-glucoronide	0.00
Estradiol-17α	0.00
Estriol	2.27
Estriol-16-glucoronide	0.00
Estrone	6.86
Estrone-3-sulfate	0.00

Compound	% Cross reactivity
11-Deoxycortisol	0.00
21-Deoxycortisol	0.00
Dihydrotestosterone	0.00
Dihydroepiandrosterone	0.00
20-Dihydroprogesterone	0.00
11-Hydroxyprogesterone	0.00
17α-Hydroxyprogesterone	0.003
17α-Pregnenolone	0.00
17α-Progesterone	0.00
Pregnanediol	0.00
Pregnanetriol	0.00
Progesterone	0.00
Testosterone	0.033
Dehydroepiandrosterone	0.00

9.2 Sensitivity

Limit of Blank (LoB)	0.028 pg/ml
Limit of Detection (LoD)	0.716 pg/ml
Limit of Quantification (LoQ)	2.364 pg/ml
Measuring range	0.716 pg/ml – 100 pg/ml
Linear range	0.919 pg/ml – 100 pg/ml

9.3 Reproducibility

9.3.1 Within-run Precision

The within-run precision was determined with 3 samples covering the complete measuring range within 20 measurements. CV was calculated as mean CV of 20 replicates.

Sample	n	Mean (pg/mL)	CV (%)
1	20	8.27	8.3
2	20	21.39	3.2
3	20	30.51	2.4

9.3.2 Between-run Precision

The between-run precision was determined with 4 samples covering the complete measuring range within 20 days in 1 run per day and with 2 replicates per run (20 x 1 x 2). CV was calculated from 40 determinations.

Sample	n	Mean (pg/ml)	CV (%)
1	40	6.60	12.0
2	40	20.44	6.5
3	40	29.88	4.9
4	40	65.01	2.8

9.3.3 Between-lot Precision

The between-lot variation was determined by 6 measurements of different samples with 3 different kit lots.

Sample	n	Mean (pg/ml)	CV (%)
1	18	1.84	8.5
2	18	12.50	12.0
3	18	7.02	10.8
4	18	51.73	9.2

9.4 Recovery

Recovery was determined by adding increasing amounts of the analyte to different patient samples containing different amounts of endogenous analyte. The percentage recoveries were determined by comparing expected and measured values of the samples.

		Sample 1	Sample 2	Sample 3
Concentration (pg/ml)		0.56	19.81	20.84
Average Recovery (%)		98.9	90.1	106.3
Range of Recovery (%)	**from**	86.6	85.4	102.4
	to	112.0	96.1	111.0

9.5 Linearity

Samples containing different amounts of analyte were serially diluted with *0,9% NaCl*. The percentage recovery was calculated by comparing the expected and measured values for the analyte.

		Sample 1	Sample 2	Sample 3
Concentration (pg/ml)		31.74	62.89	75.63
Average Recovery (%)		91.6	95.7	104.8
Range of Recovery (%)	**from**	90.0	88.5	96.0
	to	93.3	106.7	112.8

9.6 Method Comparison

A comparison of Estradiol Saliva ELISA ^{Free} (y) and LC-MS measurements (x) using clinical samples gave the following correlation:

n = 68
r = 0.988
y = 0.9659x + 0.7306

10. LIMITATIONS OF THE PROCEDURE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the instructions for use and in compliance with the laboratory quality assurance guidelines.
Any improper handling of samples or modification of this test might influence the results.

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

Substance	Conc. Range of spiked substance (ng/ml)	Mean Bias (%)
Secoisolariciresinol	0.5 – 5000	0.30
Genistein	0.5 – 5000	0.42
Daidzein	0.5 – 5000	0.27
Fulvestrant	0.5 – 5000	0.93
Coumesterol	0.5 – 5000	0.33
Dydrogesterone	10 – 1000	0.002
Pregnenolone Acetate	10 – 1000	0.002

10.3 High-Dose Hook Effect

A high-dose hook effect is not known for competitive assays.

11. LEGAL ASPECTS

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 laboratory quality assurance guidelines and 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. If there is any doubt or concern regarding a result, 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.

11.4 Reporting of Serious Incident

Any serious incident that has 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.

12. REFERENCES / LITERATURE

1. Tsang BK et al. Steroid biosynthesis by isolated human ovarian follicular cells in vitro. J Clin Endocrinol Metab 1980, 51, 1407 – 11.
2. Gore-Langton RE et al. Follicular steroidogenesis and its control. In: The physiology of Reproduction. 1988, Ed.: Knobil et al., 331-385, Raven press, New York.
3. Hall, PF. Steroid synthesis: Organization and Regulation. In: The Physiology of Reproduction. 1988, Ed.: Knobil et al., 975 – 988, Raven press, New York
4. Saldanha CJ, Remage-Healey L, and Schlinger BA. "Synaptocrine signaling: steroid synthesis and action at the synapse." Endocrine reviews 32.4, 2011, 532–549.
5. Wu CH et al. Free and protein-bound plasma estradiol-17 beta during the menstrual cycle". The Journal of Clinical Endocrinology and Metabolism. 1976, 43 (2), 436–45.
6. Fox EM et al. Novel actions of estrogen to promote proliferation: integration of cytoplasmic and nuclear pathways. Steroids, 2009, 74(7), 622-627.
7. Fernando RI et al. Estradiol abrogates apoptosis in breast cancer cells through inactivation of BAD: Ras-dependent nongenomic pathways requiring signaling through ERK and Akt. Mol Biol Cell. 2004, 15(7), 3266-3284.
8. Bovenkerk S et al. Induction of p53 expression and function by estrogen in osteoblasts. Calcif Tissue Int. 2003, 73(3), 274-280.
9. Vazquez Rodriguez G et al. Estradiol promotes breast cancer cell migration via recruitment and activation of neutrophils. Cancer Immunol Res. 2017, 5(3), 234-247.
10. Linton L et al. Associations of serum levels of sex hormones in follicular and luteal phases of the menstrual cycle with breast tissue characteristics in young women. PLoS One. 2016, 11(10), e0163865.
11. Kim JY et al. Prognostic effect of preoperative serum estradiol level in postmenopausal breast cancer. BMC Cancer. 2013, 13:503.

12. Farahani H et al. Serum and Saliva Levels of Cancer Antigen 15-3, Carcinoembryonic Antigen, Estradiol, Vaspin, and Obestatin as Biomarkers for the Diagnosis of Breast Cancer in Postmenopausal Women. Laboratory Medicine. 2020, 51, 620-627.
13. Siiteri PK et al. The serum transport of steroid hormones. Rec Progr Horm Res 1982, 38, 457 – 510
14. Martin B. et al. Binding of steroids by proteins in follicular fluid of the human ovary. J Clin Endocrinol Metab 1982, 35, 443 – 447.
15. Celec P et al. Salivary Sex Hormones during the Menstrual Cycle. Endocrine Journal 2009, 56 (3), 521-523.
16. Gandara BK, Leresche L, and Mancl L. Patterns of Salivary Estradiol and Progesterone across the Menstrual Cycle. Ann N Y Acad Sci. 2007, 1098, 446–450.
17. Baird DT 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.,1976, 125 – 33, Academic press, New York
18. McNtty KP et al. Concentration of estrogens and androgens in human ovarian venous plasma and follicular fluid throughout the menstrual cycle. J. Endocrinol. 1976, 71, 77 –85.
19. Gruhn JG, Kazer RR. Hormonal Regulation of the Menstrual Cycle: The Evolution of Concepts. Springer Science & Business Media.2013, 69–73. ISBN 978-1-4899-3496-3.
20. March CM et al. Roles of estradiol and progesterone in eliciting mid-cycle luteinizing hormone and follicle stimulating hormone surges. J. Clin. Endocrinol. Metab.1979, 49, 507 – 12.
21. Stricker R et al. Establishment of detailed reference values for luteinizing hormone, follicle stimulating hormone, estradiol, and progesterone during different phases of the menstrual cycle on the Abbott ARCHITECT analyzer". Clinical Chemistry and Laboratory Medicine. 2006, 44 (7), 883–7.
22. Simpson ER and McDonald PC. Endocrinology of pregnancy. In: Textbook of Endocrinology, Ed.: Williams R.H. 1981, 412 – 22, Saunders Company, Philadelphia.
23. Lipson, SF and Ellison, PT. Comparison of salivary steroid profiles in naturally occurring conception and non-conception cycles. Hum Reprod, 1996, 11(10), 2090-96.
24. Choe, JK, Khan-Dawood, FS, and Dawood, MY. Progesterone and estradiol in saliva and plasma during the menstrual cycle. Am J Obstet Gynecol, 1983, 147(5), 557-62.
25. Lewis JG. Steroid Analysis in Saliva: An overview. Clin Biochem Rev 2006,27 August 139-146.
26. Gameiro, CM, Romão, F, and Castelo-Branco, C. Menopause and aging: Changes in the immune system –A review. Maturitas, 2010, 67(4), 316-20.

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Use-by date	LOT	Batch code	IVD	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE marking of conformity
	Caution	REF	Catalogue number		Distributor
	Date of manufacture				