



IMMUNOASSAYS AND SERVICES

BIOGENIC AMINES & NEUROSCIENCE | ENDOCRINOLOGY | FOOD SAFETY

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Instructions for use / Gebrauchsanweisung

Estrone Saliva ELISA

REF

SA E-7100



IVD



Estrone Saliva ELISA

1. INTRODUCTION

1.1 Intended Use

The **Estrone Saliva ELISA** is an enzyme immunoassay for the quantitative *in vitro diagnostic* measurement of Estrone in saliva.

1.2 Summary and Explanation

Estrone (3-hydroxy-1,3,5 (10)-estratrien-17-one) is beside estradiol and estriol one of the three major naturally occurring estrogens. The estrogens are involved in the development of female sex organs and secondary sex characteristics. Bioassay data indicate that the estrogenic activity of estrone is considerably lower in comparison to estradiol (1). However, the physiological role of endogenous estrone is not well defined. Estrone is produced primarily from androstenedione. In premenopausal women, more than 50% of the estrone is secreted by the ovary. In prepubertal children, men and postmenopausal women, the major portion of estrone is derived from peripheral tissue conversion (2). During the follicular phase of the menstrual cycle the estrone level increases with a clear peak around day 13. The peak is of short duration and by day 16 of the cycle levels will be low again. A second peak during the luteal phase occurs around day 21 of the cycle. If fertilization does not occur production of estrone decreases again. These changes of estrone concentration are in parallel to that of estradiol (3). Until the 4th to 6th week of pregnancy, estrone originates primarily from maternal sources such as the ovaries, adrenals, or peripheral conversion thus remaining within the normal values (4). After week 6 to 10 of pregnancy the values increase gradually due to placental secretion of estrone. After menopause, estrone levels do not decline as dramatically as estradiol levels. In postmenopausal women estrone is the major estrogen. In males the concentration of E1 has been reported to rise up with age inversely to that of 17-OH-progesterone (5). In premenopausal women excessive estrone levels can result from the conversion of large amounts of androstenedione produced in polycystic ovary syndrome (6) and ovarian tumors.

2. PRINCIPLE OF THE TEST

The Estrone 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 polyclonal (rabbit) antibody directed towards a unique antigenic site of the estrone molecule.

Endogenous estrone of a patient sample competes with an estrone-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 estrone in the sample.

Having added the substrate solution, the intensity of colour developed is inversely proportional to the concentration of estrone in the patient sample.

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.

4.2 Materials required but not provided

- A microtiter plate calibrated reader (450 ± 10 nm)
- Calibrated variable precision micropipettes
- Absorbent paper
- Distilled or deionized water
- Timer
- Graph paper or software for data reduction

4.3 Storage Conditions

When stored at 2 °C – 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 – 8 °C. Microtiter wells must be stored at 2 °C – 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.

Opened kits retain activity for 8 weeks if stored as described above.

4.4 Reagent Preparation

Bring all reagents and required number of strips to room temperature prior to 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, section 13.

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).

If there is visible blood contamination the patient specimen, it should be discarded, 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

Saliva samples should be collected using a suitable collection system.

Due to the cyclic 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 5 samples always 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.

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5.2 Specimen Storage and Preparation

Saliva samples in general are stable at ambient temperature for several days.

Therefore mailing of such samples by ordinary mail without cooling will not create a problem.

The saliva samples may be stored at 2 °C to 8 °C for up to 7 days, but should be stored frozen at -20 °C as soon as possible. Longtime storage at -20 °C is possible for up to 12 months. Repeated thawing and freezing is no problem.

Each sample has to be frozen, thawed, and centrifuged at least once in order to separate the mucins by centrifugation.

Freeze samples over night at -20 °C. Then thaw samples, vortex, centrifuge for 10 minutes at approx. 10,000 × g, and carefully transfer the supernatants to fresh vials.

If a set of multiple samples is to be tested, the lab (after at least one freezing, thawing, and centrifugation cycle) has to mix 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 *Standard A* 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 sample + 90 µl *Standard A* (mix thoroughly)

b) dilution 1:100: 10 µl dilution a) 1:10 + 90 µl *Standard A* (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.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense 100 µl of each Standard, Control and samples <u>with new disposable tips</u> into appropriate wells.
3. Incubate for 30 minutes at room temperature.
4. Dispense 100 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 30 minutes at room temperature.
6. Briskly shake out the contents of the wells. Rinse the wells 5 times with 400 µl diluted <i>Wash Solution</i> 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 150 µl of Substrate Solution to each well.
8. Incubate for 15 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 <i>Stop Solution</i> .

6.3 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using scale paper or 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 Instructions for Use have been calculated automatically using a 4-Parameter curve fit. (4 Parameter Rodbard or 4 Parameter Marquardt are the preferred methods.) 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 > 333 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		Optical Units (450 nm)
Standard A	0.0 pg/ml	2.07
Standard B	3.0 pg/ml	1.81
Standard C	12.3 pg/ml	1.61
Standard D	37.0 pg/ml	1.37
Standard E	111.0 pg/ml	1.04
Standard F	333.0 pg/ml	0.63

7. EXPECTED NORMAL VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted with apparently healthy individuals, using the Estrone Saliva ELISA the following data were observed:

Population	n	Age (years)	Mean (pg/ml)	Median (pg/ml)	2.5 th – 97.5 th Percentile (pg/ml)	Range (min. – max.) (pg/ml)
Males	50	16 – 57	7.69	6.17	2.09 – 20.43	1.46 – 22.02
Females	50	19 – 58	7.37	5.04	2.61 – 23.03	1.68 – 29.25

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

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 certificate 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 0.12 pg/ml – 333.0 pg/ml.

9.2 Specificity of Antibodies (Cross Reactivity)

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

Compound	Spiked concentration (pg/ml)	% Cross-reactivity
Estrone 3-sulfate	250	52.88
Estradiol	100	8.65
Estriol	1000	0.32
Progesterone	2400	0.08
17-OH Progesterone	1000	0.05
DHEA-S	1000	0.15
Androstenedione	1000	0.09
4-Androstene-3,17-dione	250	1.66
Cortisol	30000	ND
DHEA	1440	ND
Testosterone	1000	ND
Cortisone	250	1.06
Tetrahydrocortisone	1000	0.23
Ethisterone	250	0.32

ND = none detected (< 0.08 pg/ml)

9.3 Sensitivity

The analytical sensitivity of the ELISA was calculated by subtracting 2 standard deviations from the mean of 20 replicate analyses of the *Standard A* and was found to be 0.12 pg/ml.

The Limit of Blank (LoB) is 0.08 pg/ml.

The Limit of Detection (LoD) is 1.073 pg/ml.

The Limit of Quantification (LoQ) is 3.104 pg/ml.

9.4 Reproducibility

9.4.1 Intra Assay

The within assay variability is shown below:

Sample	n	Mean (pg/ml)	CV (%)
1	10	9.28	8.5
2	10	37.85	9.4
3	10	59.17	2.4
4	10	126.80	8.8

9.4.2 Inter Assay

The between assay variability is shown below:

Sample	n	Mean (pg/ml)	CV (%)
1	30	9.64	14.1
2	30	38.57	8.2
3	30	56.58	4.2
4	30	131.11	7.1

9.5 Recovery

Recovery of the ELISA was determined by adding increasing amounts of the analyte to 4 different patient samples containing different amounts of endogenous analyte. Each sample (non-spiked and spiked) were 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)	33.60	49.19	107.21	298.68
Average Recovery (%)	97.7	97.2	107.8	96.6
Range of Recovery (%)	from	86.6	86.3	102.9
	to	107.2	110.6	112.2

9.6 Linearity

4 samples containing different amounts of analyte were serially diluted with *Standard A*. The percentage recovery was calculated by comparing the expected and measured values for the analyte.

	Sample 1	Sample 2	Sample 3	Sample 4
Concentration (pg/ml)	66.00	100.00	122.21	174.27
Average Recovery (%)	98.5	96.4	106.7	99.5
Range of Recovery (%)	from	93.9	92.8	97.8
	to	109.1	104.0	113.9

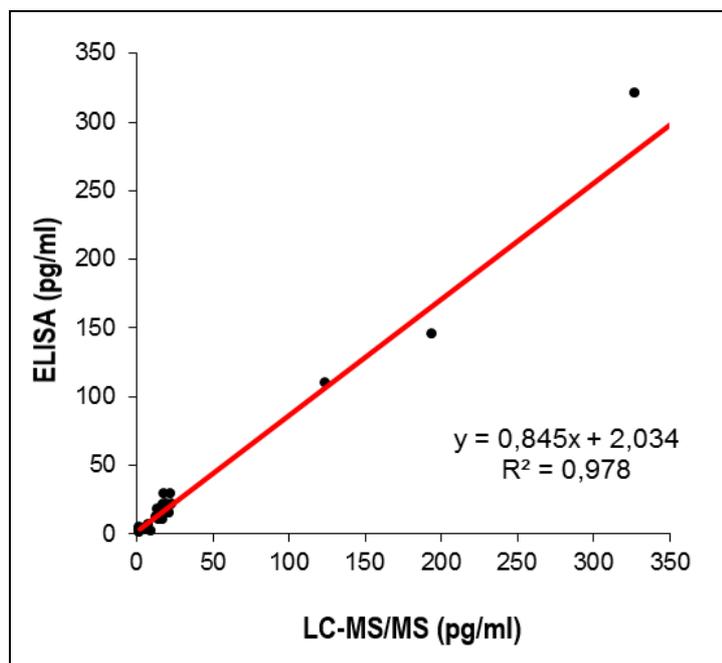
9.7 Comparison Studies

A comparison of Estrone Saliva ELISA (y) and the Reference Method LC-MS/MS (x) using clinical samples gave the following correlation:

$$n = 26$$

$$r = 0.989$$

$$y = 0.845x + 2.034$$



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.

10.1 Interfering Substances

Visible blood contamination in saliva samples will affect results.

10.2 Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of Estrone in a sample.

10.3 High-Dose-Hook Effect

No hook effect was observed in this test.

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 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

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4. Lindbert BS, Johansson EDB, Nilsson BA: Plasma levels of non-conjugated oestrone, oestradiol-17 β and oestriol during uncomplicated pregnancy. Acta Obstet Gynecol Scand 32:21, 1974.
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Symbols:

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