Instructions for use

Progesterone Saliva ELISA Free

REF **SA E-6300**   8°C 2°C Σ 96 IVD CE
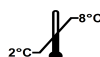
Progesterone Saliva ELISA

1. INTRODUCTION

1.1 Intended Use

Enzyme immunoassay for the in vitro diagnostic quantitative measurement of active free progesterone (a female hormone) in saliva.
Measurements obtained by this device may be used in the diagnosis and treatment of disorders of the ovaries or placenta and can be used as an aid for prediction of ovulation.

1.2 Summary and Explanation

Progesterone (4-pregnene-3, 20-dione) is a C21 steroid hormone containing a keto-group (at C-3) and a double bond between C-4 and C-5. Like other steroids, it is synthesized from cholesterol via a series of enzyme-mediated steps (1)
The steroid hormone Progesterone is a female sex hormone which, in conjunction with estrogens, regulates the accessory organs during the menstrual cycle and it is particularly important in preparing the endometrium for the implantation of the blastocyte and in maintaining pregnancy (2)
In non-pregnant women progesterone is mainly secreted by the corpus luteum whereas in pregnancy the placenta becomes the major source (3,4). Minor sources for progesterone are the adrenal cortex for both sexes and the testes for males.
The Progesterone level in saliva represents the concentration of the active free Progesterone.

2. PRINCIPLE OF THE TEST

The Progesterone Saliva ELISA Kit 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 an antigenic site of the Progesterone molecule. Endogenous Progesterone of a patient sample competes with a Progesterone-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 Progesterone in the sample. After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of Progesterone 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 the package insert 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 to 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 to 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 microtiterplate 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, BND and 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-6331   ▥ 96   **Microtiterwells**

Contents: 12 x 8 (break apart) strips, 96 wells;
Wells coated with anti-Progesterone antibody (polyclonal).

Standards and **Controls** - Ready to use

Cat. no.	Component	Standard	Concentration	Volume/Vial
SA E-6301	STANDARD A	Standard A	0 pg/ml	1 ml
SA E-6302	STANDARD B	Standard B	10 pg/ml	1 ml
SA E-6303	STANDARD C	Standard C	50 pg/ml	1 ml
SA E-6304	STANDARD D	Standard D	150 pg/ml	1 ml
SA E-6305	STANDARD E	Standard E	600 pg/ml	1 ml
SA E-6306	STANDARD F	Standard F	2400 pg/ml	1 ml
SA E-6351	CONTROL 1	Control low	Control values and ranges please refer to vial label or QC-Report.	1 ml
SA E-6352	CONTROL 2	Control high		1 ml

Conversion: pg/ml x 3.18 = pmol/l
Contents: Contain non-mercury preservative.

SA E-6340   CONJUGATE   **Enzyme Conjugate** - ready to use
Contents: Progesterone conjugated to horseradish peroxidase; Contain non-mercury preservative.
Volume: 1 x 26 ml

SA E-6355   SUBSTRATE   **Substrate Solution** - ready to use
Contents: Tetramethylbenzidine (TMB).
Volume: 1 x 25 ml

FR E-0080   STOP-SOLN   **Stop Solution** - ready to use
Contents: Contains 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** - 40X concentrated
Volume: 1 x 30 ml
see „Preparation of Reagents".

Note: Additional *Standard A* for sample dilution is available upon request.

4.2 Materials required but not provided

− A microtiter plate calibrated reader (450 nm / 620 - 630 nm)
− Calibrated variable precision micropipettes (100 µl and 200 µl)
− Absorbent paper
− Distilled water

- Timer (60 min range)
- 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 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 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.
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

Saliva can be used in this assay.

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 of 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 SALI-TUBES 100.
Do not use Salivette® tubes for sampling; 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.

At least during the luteal phase of females there is a significant episodic excretion pattern of Progesterone. Due to this episodic variation of the steroid secretion we highly recommend the strategy of multiple 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

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 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

Storage: 5 days at 2 °C to 8 °C
            at least 5 days at -20 °C, in aliquots

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

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* solution 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 *Standard A* (mix thoroughly)
b) Dilution 1:100:   10 µl of dilution a) + 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 Assay procedure

Each run must include a standard curve.

1.	Secure the desired number of coated strips in the frame holder.
2.	Dispense **100 µl** of each *Standard, Control* and **samples** <u>with new disposable tips</u> into appropriate wells.
3.	Dispense **200 µl** of *Enzyme Conjugate* into each sample and standard well. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4.	Incubate for **60 minutes** at room temperature.
5.	Briskly shake out the contents of the wells. Rinse the wells **5 times** with diluted Wash Solution (400 µl per well). Strike the inverted 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!
6.	Add **200 µl** of *Substrate Solution* to each well.
7.	Incubate for **15 minutes** at room temperature.
8.	Stop the enzymatic reaction by adding **100 µl** of *Stop Solution* to each well.
9. ⚠	Determine the absorbance (OD) of the solution in each well at **450 nm (reading) and at 620 – 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. 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 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 > 2400 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 <u>cannot</u> be used in place of data generation at the time of assay.

Standard	Optical Units (450 nm)
Standard A (0 pg/ml)	1.96
Standard B (10 pg/ml)	1.72
Standard C (50 pg/ml)	1.41
Standard D (150 pg/ml)	1.05
Standard E (600 pg/ml)	0.58
Standard F (2400 pg/ml)	0.23

7. EXPECTED NORMAL VALUES

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

In order to determine the normal range of Progesterone Saliva ELISA, saliva samples from 50 adult male and 120 female apparently healthy subjects, ages 21 to 75 years, were collected in the morning and analyzed using the Progesterone Saliva ELISA kit.
The following ranges were calculated from this study.

	Age group		Salivary progesterone pg/ml
Women	21 - 50 years	Follicular phase n = 40	19.6 - 86.5 pg/ml
	21 - 50 years	Luteal phase n = 40	99.1 - 332.6 pg/ml
	51 - 75 years	Postmenopausal n = 40	6.0 - 56.4 pg/ml
Men		n = 50	1.1 - 44.4 pg/ml

The values differ between age, new born, children, adolescents and adults.

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-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 1.1 – 2400 pg/ml.

9.2 Specificity

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

Steroid	% Cross-reactivity
Progesterone	100.0
Desoxycorticosterone	1.1
Pregnenolone	0.35
17α-Hydroxyprogesterone	0.9
Corticosterone	0.2
11-Desoxycortisol	0.1
Estriol	0.0
Estradiol 17β	0.0
Testosterone	0.2
Cortisone	< 0.1
DHEA-S	0.0
Cortisol	2.6
Androstendione	0.4
DHEA	0.0
Estron	0.0

9.3 Sensitivity

The <u>analytical sensitivity</u> of the Progesterone Saliva ELISA was calculated by subtracting 2 standard deviations from the mean of 20 replicate analyses of the Standard A and was found to be 1.1 pg/ml

9.4 Reproducibility

Intra-Assay

The within assay variability is shown below:

Sample	n	Mean (pg/ml)	CV (%)
1	20	185.7	8.1
2	20	353.6	5.5
3	20	625.3	5.3

Inter-Assay

The between assay variability is shown below:

Sample	n	Mean (pg/ml)	CV (%)
1	20	109.7	10.7
2	20	137.8	10.1
3	20	1945.8	6.7

Inter-Lot

The inter-assay (between-lots) variation was determined by repeated measurements of 3 samples in 3 different kit lots.

The between lot variability is shown below:

Sample	n	Mean (pg/ml)	CV (%)
1	18	196.6	5.4
2	18	346.9	3.4
3	18	612.4	3.9

9.5 Recovery

Recovery of the Progesterone Saliva ELISA was determined by adding increasing amounts of the analyte to three different saliva samples containing different amounts of endogenous analyte. Each sample (native 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
Concentration (pg/ml)		28.6	261.0	378.0
Average Recovery (%)		102.8	98.6	99.7
Range of Recovery (%)	from	89.1	93.8	93.6
	to	113.1	107.5	105.1

9.6 Linearity

In total six saliva samples containing different amounts of analyte were serially diluted with Standard A and assayed with the Progesterone Saliva ELISA.

Three of these samples were serially diluted directly, and the other 3 samples at first were spiked with progesterone and then serially diluted up to 1:128.

The percentage recovery was calculated by comparing the expected and measured values for progesterone. Samples above this range must be diluted and re-run.

		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Concentration (pg/ml)		130.5	321.0	378.7	501.6	580.0	1000.0
Average Recovery (%)		98.9	107.1	104.6	100.0	98.0	90.6
Range of Recovery (%)	from	85.7	102.9	95.3	89.3	86.9	86.0
	to	111.6	112.1	109.8	105.3	109.7	97.3

9.7 Comparison Studies

A study was performed that evaluated 24 saliva samples collected from adult men and women. The samples were analyzed with the Progesterone Saliva ELISA and a LC-MS/MS mass spectrometer to determine the concentration of free progesterone in the saliva samples. A correlation of 0.997 and regression formula of $y = 0.9612x - 11.071$ were obtained.
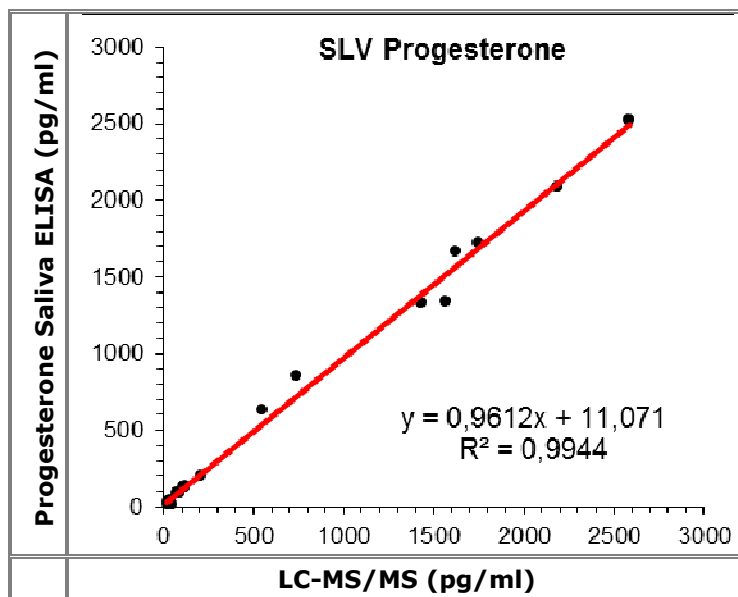
10. LIMITATIONS OF PROCEDURE

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

Blood contamination ≥ 0.16% in saliva samples will affect results, and usually can be seen by eye. Concentrations of Sodium Azide ≥ 0.02% interferes in this assay and may lead to false results.

10.2 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

1. Miller W.L. (1988): Molecular biology of steroid hormone synthesis,
   Endocrin. Rev. 9, 295-318
2. Filicori M., Butler J.P., Crowley W.F. Jr. (1984): Neuroendocrine regulation of the corpus luteum in the human,
   J. Clin. Invest. 73, 1638
3. Csapol Al., Pulkkinen M. O., Wiest W.G. (1973): Effects of lutectomy and progesterone replacement therapy in early pregnancy patients.
   Am J. Obstet. Gynecol. 115, 759
4. Henson M.C. (1998): Pregnancy maintenance and the regulation of placental progesterone biosynthesis in the baboon.
   Human Reproduction Update, 4, 389-405

Symbols:

+8/+2 °C	Storage temperature	▲▲	Manufacturer	∇ (Σ)	Contains sufficient for <n> tests
(hourglass)	Expiry date	LOT	Batch code	I V D	For in-vitro diagnostic use only!
(consult book) i	Consult instructions for use	CONT	Content	CE	CE labelled
⚠	Caution	REF	Catalogue number		