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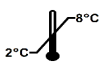
Instructions for use

Progesterone ELISA

REF **FR E-2500**



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Progesterone ELISA

1. INTRODUCTION

1.1 Intended Use

The **Progesterone ELISA** is an enzyme immunoassay for the quantitative ***in vitro diagnostic*** measurement of Progesterone in serum or plasma (EDTA, lithium heparin or citrate plasma).

1.2 Summary and Explanation

Progesterone (pregn-4-ene-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 (1-4).
This steroid hormone 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. In non-pregnant women progesterone is mainly secreted by the corpus luteum whereas in pregnancy the placenta becomes the major source. Minor sources are the adrenal cortex for both sexes and the testes for males.
Progesterone circulates in blood mainly bound to Corticosteroid Binding Globulin (CBG), Sex Hormone Binding Globulin (SHBG) and Albumin. Only 2 – 10% of the total concentration circulates as free hormone. Blood progesterone concentrations vary widely according to the phases of menstrual cycle; they are lower than 1 ng/ml (3.2 nmol/l) in the follicular phase and around 10 – 20 ng/ml (32 – 64 nmol/l) in the luteal phase. The maximal levels are achieved 4 – 7 days after ovulation and remain elevated for 4 – 6 additional days prior to falling to the preovulatory levels 24 hours before the onset of menstruation.
Since the rise and fall of progesterone parallel the activity of ovarian follicle and corpus luteum, measurements of plasma progesterone are clinically used to confirm ovulation and normal function of the corpus luteum in non-pregnant women.
If ovulation does not occur the corpus luteum is not formed and no cyclical rise of progesterone in plasma is observed. Abnormal progesterone secretion has been implicated in premenstrual tension, irregular shedding of endometrium, dysmenorrhoea, and luteal insufficiency.
Progesterone concentration can vary not only from subject to subject but also in the same person from day to day or even from hour to hour. Consequently, in gynaecological disorders or abnormal pregnancies serial measurements rather than single ones are recommended for a proper interpretation of results.
During pregnancy progesterone is widely produced by placenta, and plasma levels rise steadily achieving values as high as 200 ng/ml at term.

2. PRINCIPLE OF THE TEST

The Progesterone 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 Progesterone molecule.
During the first incubation, the progesterone in the added sample competes with the added enzyme conjugate, which is a progesterone molecule 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 abruptly 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

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 (20 °C – 26 °C) before starting the test. Temperature will affect the optical density 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

FR E-2531 **MTP 96** **Microtiterwells**

Content: 12x8 (break apart) strips, 96 wells;
Wells coated with anti-progesterone antibody (polyclonal).

Standards – ready to use

Cat. no.	Component	Standard	Concentration	Volume/Vial
FR E-2501	**STANDARD A**	Standard A	0 ng/ml	1 ml
FR E-2502	**STANDARD B**	Standard B	0.3 ng/ml	1 ml
FR E-2503	**STANDARD C**	Standard C	1.25 ng/ml	1 ml
FR E-2504	**STANDARD D**	Standard D	2.5 ng/ml	1 ml
FR E-2505	**STANDARD E**	Standard E	5 ng/ml	1 ml
FR E-2506	**STANDARD F**	Standard F	15 ng/ml	1 ml
FR E-2507	**STANDARD G**	Standard G	40 ng/ml	1 ml

Content: Contain non-mercury preservative.

Conversion: 1 ng/ml = 3.18 nmol/l

FR E-2540 **CONJUGATE** **Enzyme Conjugate** – ready to use

Content: Progesterone conjugated to horseradish peroxidase;
Contains non-mercury preservative.

Volume: 1 x 25 ml

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

Content: Tetramethylbenzidine (TMB).

Volume: 1 x 25 ml

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

Content: 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

FR E-0030	**WASH-CONC** **40x**	**Wash Solution** – 40X concentrated
Volume:	1 x 30 ml	
	See „Reagent Preparation"	

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

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
- Absorbent paper
- Distilled 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 (20 °C – 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.
The diluted Wash Solution is stable for 1 week 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

Serum or plasma (EDTA, lithium-heparin- or citrate plasma) can be used in this assay.

Note: Samples containing sodium azide should not be used in the assay.
In general it should be avoided to use haemolytic, icteric or lipaemic specimens. For further information refer to chapter "Interfering Substances".

5.1 Specimen Collection

Serum:
Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Plasma:
Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

5.2 Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 7 days at 2 °C – 8 °C prior to assaying.
Specimens held for a longer time (up to 12 months) should be frozen only once at -20 °C prior to assay. Thawed samples 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* 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.
- Optical density 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 **25 µl** of each ***Standard, Control*** and **sample** with new disposable tips into appropriate wells.
3.	Incubate for **5 minutes** at room temperature.
4.	Dispense **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 **60 minutes** at room temperature.
6.	Rinse the wells **3 times** with 400 µl diluted Wash Solution per well, if a plate washer is used – or – Briskly shake out the contents of the wells. Rinse the wells **3 times** with 300 µl diluted *Wash Solution* per well for manual washing. 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 **200 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 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. Calculate the average optical density (OD) values for each set of standards, controls and patient samples.
2. Using linear 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.
3. Using the mean OD 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 > 40 ng/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 ng/ml)	1.52
Standard B (0.3 ng/ml)	1.17
Standard C (1.25 ng/ml)	0.88
Standard D (2.5 ng/ml)	0.69
Standard E (5.0 ng/ml)	0.55
Standard F (15 ng/ml)	0.35
Standard G (40 ng/ml)	0.13

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 subjects, using the Progesterone ELISA the following data were observed:

Population		n	Mean (ng/ml)	Median (ng/ml)	2.5th – 97.5th Percentile (ng/ml)	Range (min. – max.) (ng/ml)
Males		49	0.36	0.34	0.05 – 0.92	0.05 – 0.94
Females						
	Follicular Phase	35	0.79	0.76	0.21 – 1.72	0.21 – 1.80
	Luteal Phase	45	12.89	13.00	3.78 – 24.60	2.90 – 27.10
	Postmenopausal	28	0.53	0.59	0.18 – 0.83	0.15 – 0.84

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 0.140 ng/ml – 40.0 ng/ml.

9.2 Specificity of Antibodies (Cross-Reactivity)

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

Steroid	Cross-Reaction (%)
Progesterone	100.00
17α OH Progesterone	0.30
Estriol	< 0.10
Estradiol 17β	< 0.10
Testosterone	< 0.10
11-Desoxycorticosterone	1.10
DHEA-S	< 0.02
Cortisol	< 0.02
Corticosterone	0.20
Pregnenolone	0.35
Cortison	< 0.10
11-Desoxycortisol	0.10

9.3 Sensitivity

The analytical sensitivity of the ELISA was calculated by subtracting 2 standard deviations to the mean of 20 replicate analyses of the *Standard A* and was found to be 0.045 ng/ml.

The Limit of Blank (LoB) is 0.120 ng/ml.
The Limit of Detection (LoD) is 0.140 ng/ml.
The Limit of Quantification (LoQ) is 0.144 ng/ml.

9.4 Reproducibility

9.4.1 Intra Assay

The within assay variability is shown below:

Sample	n	Mean (ng/ml)	CV (%)
1	20	0.6	5.4
2	20	4.7	7.0
3	20	10.8	6.9

9.4.2 Inter Assay

The between assay variability is shown below:

Sample	n	Mean (ng/ml)	CV (%)
1	12	0.6	10.0
2	12	4.6	4.3
3	12	10.7	5.6

9.4.3 Inter-Lot

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

Sample	n	Mean (ng/ml)	CV (%)
1	18	1.2	7.2
2	18	38.7	3.1

9.5 Recovery

Samples have been spiked by adding progesterone solutions with known concentrations.
The recovery (%) was calculated by multiplying the ratio of measured and expected values with 100.

		Sample 1	Sample 2	Sample 3
Concentration (ng/ml)		1.6	4.2	11.0
Average Recovery (%)		101.9	104.1	97.0
Range of Recovery (%)	from	97.8	96.3	90.9
	to	112.0	109.0	105.6

9.6 Linearity

Samples were measured undiluted and in serial dilutions with *Standard A*. The recovery (%) was calculated by multiplying the ratio of expected and measured values with 100.

		Sample 1	Sample 2	Sample 3
Concentration (ng/ml)		1.6	4.2	11.0
Average Recovery (%)		102.5	99.1	106.5
Range of Recovery (%)	from	92.0	87.8	104.7
	to	111.9	110.3	108.6

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

Haemoglobin (up to 4 mg/ml), Bilirubin (up to 0.5 mg/ml) and Triglyceride (up to 1.8 mg/ml) have no influence on the assay results.

10.2 Drug Interferences

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

10.3 High-Dose-Hook Effect

A High-Dose-Hook Effect is not relevant 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 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. Filicori M, Butler JP, Crowley WF Jr. Neuroendocrine regulation of the corpus luteum in the human. J Clin Invest. 73:1638 1984.
2. Katt JA, Duncan JA, Herbon L, et al. The frequency of gonadotropin releasing hormone stimulation determines the number of pituitary gonadotropin releasing hormone receptors. Endocrinology 1985; 116:2113.
3. Csapo AI, Pulkkinen MO, Wiest WG: Effects of lutectomy and progesterone replacement therapy in early pregnancy patients. Am J Obstet Gynecol 115:759, 1973.
4. Thomas: Labor und Diagnose; TH-Books-Verlags-Gesellschaft; 8. Auflage (2012)

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 marking of conformity
	Caution	REF	Catalogue number		Distributor
	Date of manufacture				