





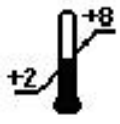













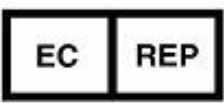










Progesterone

**Enzyme Immunoassay for Quantitative Determination of
Progesterone in Human Serum**

Instructions for use

1. SYMBOL LEGEND

	In vitro diagnostic medical device		EC Declaration of conformity
	Catalogue number		Batch code
	Temperature limitation		Biological risks
	Caution, consult accompanying documents		Conjugate
	Coated microplate (96 wells)		Substrate
	Wash solution, 20X concentrated		Stop solution
			Optical density
	Calibrators		Contains sufficient for <n> tests
	Control		Deionized or distilled water
	Authorized Representative in the European Community		Trial
	Reconstitute with specified volume of liquid		
	Corrosive		Irritant
	Date of manufacture		Manufacturer
	Use by		

2. INTENDED USE

Progesterone kit is provided for the **quantitative** determination of progesterone in human serum.

Progesterone is a steroid hormone with a molecular mass of 314 Da. The quantitative measurement of serum progesterone is a valuable tool for evaluation of corpus luteus functional status. It is also used in pregnancy surveillance and may be performed for investigational purposes.

3. PRINCIPLE OF TEST

Progesterone kit is a competitive solid phase enzyme immunoassay. During the incubation progesterone of the tested samples and horseradish peroxidase (HRP) labeled Progesterone bind to the antibodies coated onto the inner surface of the microplate wells until the balance between them occurs. Separation of free and bound to antibodies progesterone as well as conjugate progesterone - peroxidase occurs while extracting the contents of the wells. The amount of bound conjugate is inversely proportional to the quantity of progesterone in the sample (Fig. 1).

During the incubation with TMB solution the colour is developing. The intensity of the colour is inversely proportional to the progesterone concentration in specimens. Progesterone concentration in the patient sample is read from a standard curve that is processed in each assay.

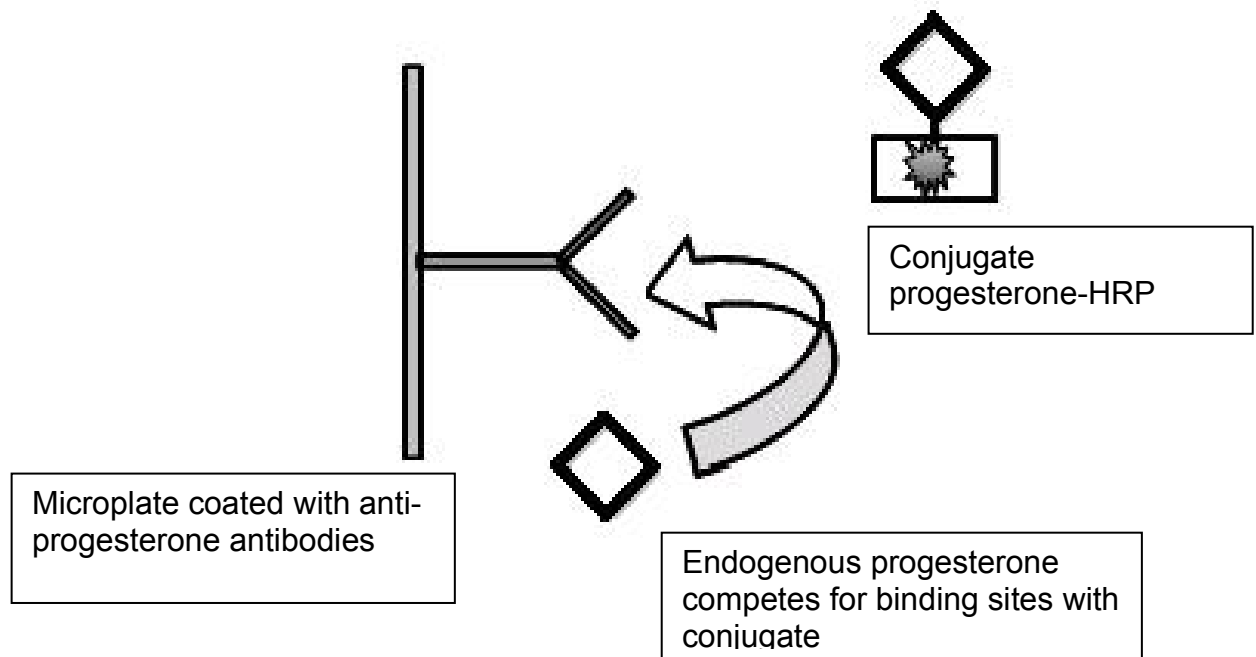


Fig. 1. Assay scheme

4. STORAGE AND STABILITY OF THE KIT

Expiration date of the kit is printed on the box label; expiration date for each component is printed on the respective label.

Progesterone kit should be stored at 2-8°C upon receipt, preferably in the original kit box, until the expiration date. Storage at 25°C is allowed but for no more than 5 days.

Shelf life of the kit is 12 months.

After initial opening the kit is stable for 1 month if stored at 2-8°C.

If used in several separate experiments, kit contents should be stored as follows:

unused strips: in a firmly closed resealable zipper bag at 2-8°C until the expiration date;

vials with conjugate, calibrators and control (ready-to-use

or reconstituted), concentrated wash solution, stop solution: at 2-8°C for no more than 1 month after opening;

opened vial with substrate: at 2-8°C for no more than 1 month after opening, protected from light;

opened vials with concentrated Trial, concentrated wash solution and stop solution: at 2-8°C until the expiration date;

wash solution and Trial solution prepared for use: at room temperature (18-25°C) for no more than 5 days, in a firmly closed bottle.

Damaged Test Kits

In case of any severe damage of the test kit or components, it has to be informed in writing, during one week after receiving the kit. Usage of severely damaged single components for a test run is not recommended.

5. SAMPLE COLLECTION AND STORAGE

Collect blood by venipuncture. After clotting, the serum is separated by centrifugation.

Do not use plasma, haemolyzed (bright red) or lipemic (milky) serum samples as well as samples containing sodium azide as preservative.

Store serum samples at 2-8°C for no more than 2 days. Aliquot and freeze samples for longer storage (-20°C and lower). Avoid repeated freezing.

6. EXPECTED VALUES

Serum samples collected between 9 and 11 a.m. from 60 apparently healthy people (both males and females) between the ages of 21–45, were assayed with Progesterone kit. The results are listed below. These limits should be considered as guidelines only.

Group	n	Progesterone concentration range (nmol/L)
<i>Female</i>	60	
Follicular phase		<0.5-6
Luteal phase		10-89
<i>Male</i>		<0.5-5.2

It is highly recommended each laboratory to determine its own reference range of progesterone concentrations.

7. QUALITY CONTROL

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.

8. REAGENT PREPARATION

Allow all the reagents to reach room temperature (18-25°C), and then thoroughly stir.

MP Keep **microplate** at room temperature for at least 30 minutes before opening the bag. Place required number of strips onto strip holder. Place unused strips into the resealable zipper bag and reseal duly.

CAL CONTROL Calibrators and Control

Liquid calibrators and control are ready to use.

Prepare lyophilized calibrators and control as follows. Gently tap on the vial caps to knock off all the dry matter. Open the vials and carefully place the caps upside down on the clean dry surface. Add 0.5 mL of distilled or deionized water to each vial with lyophilized calibrators and control, close vials with the corresponding caps and leave for 10 min at room temperature (18-25°C) without stirring. Then stir gently avoiding foaming, until the dry matter is completely dissolved. Leave for another 10 minutes at room temperature stirring gently periodically. Make sure that no dry matter is left on the caps and walls of the vials.

WASH P Prepare required volume of **wash solution** by dilution of the concentrate 20-fold with distilled or deionized water. For example:

5 mL of **WASH P** **20X** + 95 mL of water.

Mix thoroughly, avoid foaming.

SUB Protect **substrate** from direct light.

9. SAMPLE PREPARATION

Allow samples to reach room temperature. Stir samples gently in order to ensure homogeneity.

10. PERFORMANCE CHARACTERISTICS OF THE ASSAY

10.1. Calibration-Traceability

Progesterone kit was calibrated against the Working Standard, which had been manufactured by gravimetric method based on weighing purified synthetic progesterone into analyte-free matrix.

10.2. Specificity

Cross-reaction of anti-progesterone monoclonal antibodies with different steroids is shown below.

Steroid	Cross-reaction, %
Progesterone	100
5- α -pregnan-3,20-dione	10.5
17-hydroxyprogesterone	1.0
Corticosterone	0.01
Pregnenolone	0.90
11-Deoxycorticosterone	0.30
11-Deoxycortisol	0.03
Cortisol	0.005

10.3. Analytical Sensitivity

Analytical sensitivity of **Progesterone kit**, i.e. concentration, that can be distinguished from zero calibrator is 0.5 nmol/L. It was defined as mean of 10 replicates of calibrator 0 minus 2 SD.

10.4. Measurement Range

Progesterone kit was validated for measurement of progesterone concentration within the concentration diapason of 0.5–100 nmol/L (for manual test) and 0.5 - 300 nmol/L (for analyser “Alisei”).

10.5. Measurement Units

In **Progesteron kit** the concentrations of calibrators are specified in nmol/L. To convert into ng/mL, multiply the concentration in nmol/L by 0.314.

10.6. Intra- and Inter-Assay Variation (Precision)

For **intra-assay CV** determination, 8 serum samples were run, each in 9 replicates. The results are shown below.

Sample	Mean progesterone concentration, nmol/L	Intra-assay CV	
		SD	CV, %
HS 1	2.3	0.20	8.8
HS 2	4.5	0.34	7.5
HS 3	7.5	0.34	4.5
HS 4	12.4	0.26	2.1
HS 5	19.3	0.54	2.8
HS 6	26.3	0.88	3.3
HS 7	59.3	2.37	4.0
HS 8	59.0	2.24	3.8

For **inter-assay CV** determination, 5 serum samples were assayed 3 times by different operators with 1-week interval. Each specimen was run in 9 replicates. The results are shown below.

Sample	Mean progesterone concentration, nmol/L			Inter-assay CV	
	1 assay	2 assay	3 assay	SD	CV, %
HS 1	1.2	1.5	1.3	0.15	11.5
HS 2	3.7	4.4	4.0	0.35	8.7
HS 3	13.2	15.2	14.1	1.00	7.1
HS 4	44.2	46.1	48.4	2.10	4.5
HS 5	52.0	55.5	58.7	3.35	6.0

11. LIMITATION OF THE METHOD

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. For diagnosis establishment, a physician is supposed to consider all available clinical and laboratory findings.

12. SAFETY PRECAUTIONS

This kit is for in vitro diagnostic use only. Operator should follow the manual closely in order to ensure reliable data. The manual is valid for the present kit only, within the listed composition. Any substitution of kit components is not allowed by CE regulations.

Do not use kit or its components after the expiration date indicated on the label. Take into account stability period for reconstituted reagents.

Do not mix or use together reagents from different lots of the kit except substrate, stop solution and wash solution.

Do not use substrate, stop solution and wash solution supplied by other vendors.

Use only "P"-labeled wash solution.


Note that stop solution is 1N HCl solution. Avoid contacts with skin and mucosa. In case of contact rinse affected area thoroughly with plenty of water and seek medical advice.


Take into account the following common procedural notes:

- always pipette reagents into wells immediately after washing procedure;
- avoid contamination of the solutions;
- in case of partial use of the kit, dispense only required volume of the reagent into the tray;


- do not pour unused reagents back into the original vials;
- avoid exposure to direct sunlight during incubations;
- always pipette reagents in the same order to minimize reaction time differences between wells; the total dispensing time for the calibrators, control and samples must not exceed 15 min;
- the incubation temperature for all the immunological reactions must be kept at 37°C;
- do not touch the bottom of the wells;
- calibrators should be measured in each separate assay. It is also recommended to measure each time progesterone concentration in the control.


TMB solution should be colourless. Light colouring of solution is admissible. Avoid direct exposure of substrate to sunlight.

 Source materials of human origin used for kit components preparation were tested and found negative for HBsAg, anti-HIV and anti-HCV antibodies. However, none of known laboratory test guarantees absence of these viral agents. Therefore, all kit components and patient's samples should be handled as potentially hazardous.

 After usage strips, calibrators, control, specimens, and all consumables which contacted with specimens during handling, storage or assay (tubes, vials, gloves, pipette tips etc.) should be collected separately and sterilized by autoclaving. Instead of autoclaving pipette tips may be sterilized by disinfectant treatment. After sterilization all components and expendable materials may be utilized as non-dangerous

garbage. Other components of the kit should be discarded into conventional garbage.

 During manual washing procedure do not discard the contents of the wells directly to drainage. Use a container with disinfectant solution.



 As the kit contains potentially hazardous material, the following precautions should be taken:

- do not smoke, eat or drink while performing the assay;
- always use protective gloves;
- never pipette material by mouth;
- in case of spilling, wipe up the spills promptly and wash affected area thoroughly using decontaminant.

GLP including all general and individual regulations should be applied for the kit usage.

13. MANUAL TEST (REF 100-02)

13.1. Material Provided

MP	Microplate: 12 breakable 8-well strips (total 96 wells) coated with anti-Progesterone monoclonal antibodies	1 pcs
CONJ	Conjugate: containing progesterone conjugated with HRP	18 mL, ready to use
0-5 CAL	Calibrators: serum-based solution or lyophilized preparations containing known progesterone concentrations - 0; 1; 3; 10; 30; 100 nmol/L. For exact progesterone concentrations, see vial labels 	6 vials, 0.5 mL each; ready to use or lyophilized preparations
CONTROL	Control: serum-based solution or lyophilized preparation containing known progesterone concentration. For exact range of progesterone concentration see vial label 	0.5 mL, ready to use or lyophilized preparation
SUB	Substrate (TMB solution): 3,3',5,5'-tetramethylbenzidine solution in citrate buffer containing hydrogen peroxide	14 mL, ready to use
WASH P 20X	Wash solution P, 20X concentrated: surfactant in buffered saline, sufficient for preparation of 280 mL of solution	2 X 14 mL, concentrated
STOP	Stop solution: 1N HCl solution	14 mL, ready to use

13.2. Equipment and Materials Required but not provided

1-channel calibrated variable precision pipettes, with disposable tips;

8-channel calibrated variable precision pipette, with disposable tips;

microplate incubator/shaker (37°C, shaking speed 400–800 rpm);

manual or automatic equipment for rinsing wells;

calibrated microplate reader (450 nm);

vortex tube mixer;

deionized or distilled water;

graduated beaker and cylinder of appropriate volume;

latex or plastic gloves;

trays for pipetting reagents with 8-channel pipette;

disinfectant;

absorbent material (for manual wash).

13.3. Test procedure

Progesterone kit is designed for 96 tests. This is sufficient for 40 unknowns, 6 calibrators, 1 control and 1 blank (OD of TMB solution) in duplicates, provided that all the strips are used simultaneously.

13.3.1. Assay Procedure

(See assay scheme, section 13.5)

A. Pipette:

20 μ L of calibrators **CAL** (0-5), control **CONTROL** and patient's samples in duplicates into the respective wells;

Leave wells A1-A2 empty for blank!

B. Pipette:

150 μ L of conjugate **CONJ** into each well, **except wells A1-A2.**

C. Incubate strips for **1 hour while shaking (500–800 rpm) at 37°C.**

D. Wash 4 times, as described in section 13.3.2.

E. Pipette **100** μ L of substrate **SUB** into each well (including blank); incubate **at room temperature (18-25°C) in the dark for 15-30 minutes**, depending on the colour intensity, or **10 minutes while shaking (500-800 rpm) at 37°C.**

F. Pipette **100** μ L of stop solution **STOP** into each well (including blank) in the same sequence and at the same speed as used for dispensing TMB substrate. Shake for 1–2 min at room temperature.

G. Read OD at **450 nm within 20 min.**

13.3.2. Wash Procedure

It is advisable to use an automatic microplate washer set at 4 wash cycles and a volume of 300 μL of wash solution per well per cycle.

If an automatic washer is not available, the wash procedure can be carried out manually as follows:

remove the contents of the wells into a container with disinfectant;

dispense 300 μL of wash solution, prepared according to section 8, into each well, shake the plate carefully for 5–10 sec and remove the contents of the wells; repeat 4 times;

strike the wells sharply on absorbent material to remove any liquid residue.

13.4. Data Processing

If the reader cannot be adjusted to zero using the substrate blank in wells A1-A2, subtract mean OD value of wells A1-A2 from all OD values before further calculations.

Example:

OD (Cal 0) measured = 2.28 and OD (blank) = 0.06;

OD (Cal 0) calculated = $2.28 - 0.06 = 2.22$

13.4.1. Data Reliability (for OD Measured at 450 nm)

The data should meet the following criteria:

average blank OD (in wells A1-A2) ≤ 0.100 ;

average OD of Cal 0 ≥ 1.500 (after blank subtraction);

control's concentration must fall within the acceptability range that is shown on the vial label.

If the data obtained do not meet the criteria, the results are considered unreliable and the test should be repeated.

13.4.2. Quantitative Determination

Specialized software for quantitative determination is recommended. Mean OD of calibrators are plotted versus their respective progesterone concentrations using 4PL fit (see typical standard curve, fig. 2). Calculate concentration of progesterone in samples using standard curve.

Any extrapolation of the standard curve to progesterone above the nominal value of the Calibrator 5 is forbidden.

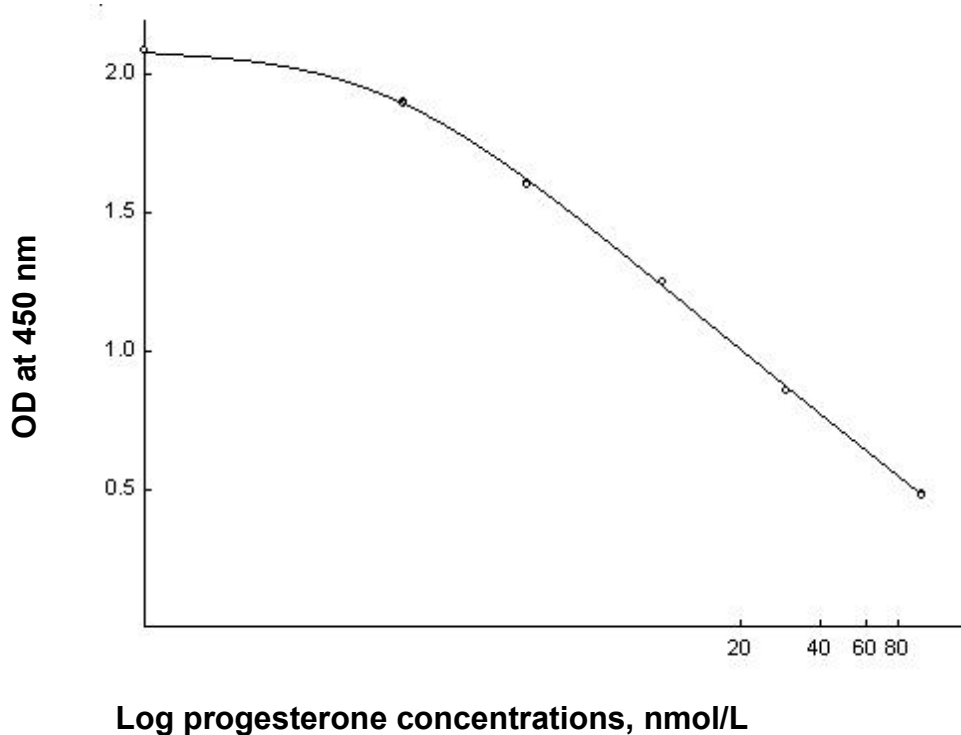


Fig. 2. Example of typical standard curve.

Do not use for evaluation of real assay data!

13.5. Assay scheme

Reagents	Wells	«Blank»	CAL	Samples
			CONTROL	
CAL		–	20 µL	–
CONTROL		–	–	20 µL
Samples		–	–	20 µL
CONJ		–	150 µL	150 µL
Incubation No. 1	60 min, 37°C, 500–800 rpm			
WASH P (diluted)	4 x 300 µL			
SUB	100 µL	100 µL	100 µL	100 µL
Incubation No. 2	15–30 min, 18-25°C, in the dark			
	10 min, 37°C, 500–800 rpm			
STOP	100 µL	100 µL	100 µL	100 µL
Stirring	1–2 min, 18-25°C			
OD measuring	450 nm			
Calculations	Corresponding software			

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