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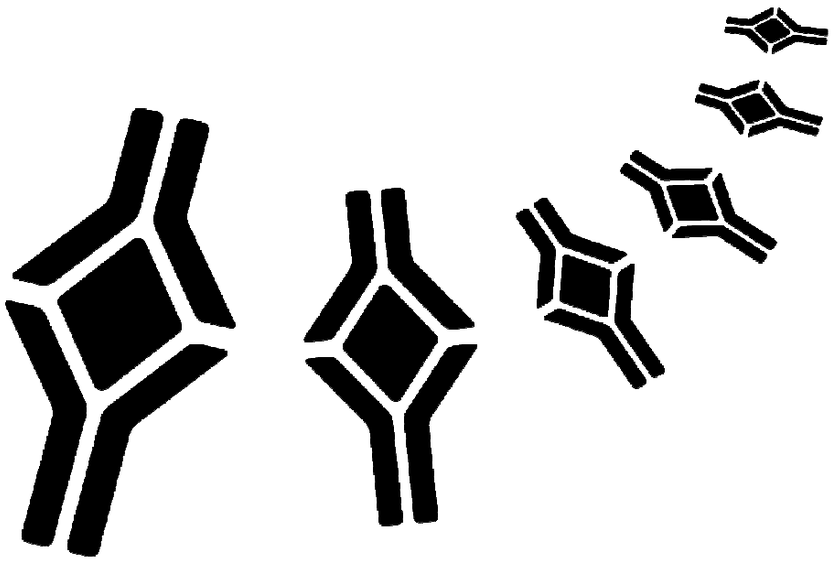

PROGESTERONE ELISA

Product Data Sheet

Cat. No.: RCAN-P-305R

For Research Use Only

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This kit is manufactured by:
BioVendor – Laboratorní medicína, a.s.

Use only the current version of Product Data Sheet enclosed with the kit!

1. INTENDED USE

For the direct quantitative determination of Progesterone by enzyme immunoassay in human serum. For *in vitro* diagnostic use only.

2. PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabeled antigen (present in standards, control and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed is inversely proportional to the concentration of progesterone in the sample. A set of standards is used to plot a standard curve from which the amount of progesterone in patient samples and controls can be directly read.

3. CLINICAL APPLICATIONS

Progesterone is a C-21 female sex steroid hormone with a variety of physiological effects. In the follicular phase of the menstrual cycle, progesterone is produced in low levels. It increases to the LH peak and then sharply rises 3 to 4 days later to higher levels, remaining elevated through the 10th to 12th days after the LH peak. Then there is a sharp decline to the low levels of the follicular phase. It is responsible for the induction of the cyclic changes in the endometrium of the uterus allowing implantation and successful growth of the fertilized ovum and maintenance of pregnancy.
Progesterone measurements are used in documenting ovulation and in the management of difficulties during the first trimester of pregnancy. Levels of progesterone may be useful in the evaluation of sterility due to luteal phase defects, prediction of impending abortion, and the diagnosis of ectopic pregnancy.
Normal values of progesterone may be affected by drugs such as, oral contraceptives, superovulatory drugs, estrogen replacement therapy medication, and GnRH analogues. The removal of ovarian function following surgical oophorectomy or chemotherapy may influence serum progesterone values.

4. PROCEDURAL CAUTIONS AND WARNINGS

1. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
2. Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
5. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
6. A calibrator curve must be established for every run.
7. The control should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the control do not reflect established ranges.
9. When reading the microplate, the presence of bubbles in the microwells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
10. The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
11. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
13. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
14. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

5. LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of progesterone in human serum. The kit is not calibrated for the determination of progesterone in saliva, plasma or other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only calibrator A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
5. The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.

6. SAFETY CAUTIONS AND WARNINGS

6.1 Potential Biohazardous Material

Human serum that may be used in the preparation of the standards and control has been tested and found to be non-reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. However no test method can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

6.2 Chemical Hazards

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

7. SPECIMEN COLLECTION AND STORAGE

Approximately 0.1 ml of serum is required per duplicate determination. Collect 4-5 ml of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

8. SPECIMEN PRETREATMENT

This assay is a direct system; no specimen pretreatment is necessary.

9. REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 25, 50, 100, 150 and 300 µl
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Microwell plate reader with a filter set at 450nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10).

10. REAGENTS PROVIDED

10.1 Rabbit Anti-Progesterone Antibody Coated Microwell Plate-Break Apart Wells- Ready To Use.

Contents: One 96 well (12x8) polyclonal antibody-coated microwell plate in a resealable pouch with desiccant.
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

10.2 Progesterone -Horseradish Peroxidase (HRP) Conjugate Concentrate – X100
Contents: Progesterone-HRP conjugate in a protein-based buffer with a non-mercury preservative.
Volume: 400 µl/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.
Preparation: Dilute 1:100 in assay buffer before use (eg. 20 µl of HRP in 2 ml of assay buffer). If the whole plate is to be used dilute 120 µl of HRP in 12ml of assay buffer. Discard any that is left over.

10.3 Progesterone Calibrators - Ready To Use.
Contents: Six vials containing progesterone in a human serum-based buffer with a non-mercury preservative. Prepared by spiking serum with a defined quantity of progesterone.
*Listed are approximate concentrations, please refer to the Certificate of Analysis for exact concentrations.

Calibrator	Concentration	Volume/Vial
Calibrator A	0 ng/mL	2.0 mL
Calibrator B	0.3 ng/mL	0.5 mL
Calibrator C	1 ng/mL	0.5 mL
Calibrator D	5 ng/mL	0.5 mL
Calibrator E	20 ng/mL	0.5 mL
Calibrator F	60 ng/mL	0.5 mL

Storage: Refrigerate at 2-8°C
Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

10.4 Control - Ready To Use.
Contents: One vial containing progesterone in a human serum-based buffer with a non-mercury preservative. Prepared by spiking serum with a defined quantity of progesterone. Refer to the Certificate of Analysis for expected value and acceptable range.
Volume: 0.5 ml/vial
Storage: Refrigerate at 2-8 °C
Stability: 12 months in unopened vial or as indicated on label. Once opened, the control serum should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

10.5 Wash Buffer Concentrate - X10
Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Volume: 50 ml/bottle
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.
Preparation: Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 ml of the wash buffer concentrate in 450 ml of water.

10.6 Assay Buffer - Ready To Use*.
Contents: One vial containing a protein-based buffer with a non-mercury preservative.
Volume: 15 ml/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

10.7 TMB Substrate - Ready To Use.
Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
Volume: 16 ml/bottle
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

10.8 Stopping Solution - Ready To Use.
Contents: One vial containing 1M sulfuric acid.
Volume: 6 ml/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

11. ASSAY PROCEDURE

Specimen Pretreatment:
None.
All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Prepare working solutions of the progesterone HRP conjugate and wash buffer.
2. Remove the required number of microwell strips. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette 25 µl of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
4. Pipette 100 µl of the conjugate working solution into each well (We recommend using a multichannel pipette).
5. Incubate on a plate shaker (approximately 200 rpm) for 1hour at room temperature.
6. Wash the wells 3 times with 300 µl of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry (The use of a washer is recommended).
7. Pipette 150 µl of TMB substrate into each well at timed intervals.
8. Incubate on a plate shaker for 10-20 minutes at room temperature (or until calibrator A attains dark blue colour for desired OD).
9. Pipette 50 µl of stopping solution into each well at the same timed intervals as in step 7.
10. Read the plate on a microwell plate reader at 450nm within 20 minutes after addition of the stopping solution.

* If the OD exceeds the upper limit of detection or if a 450nm filter is unavailable, a 405 or 415nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.

12. CALCULATIONS

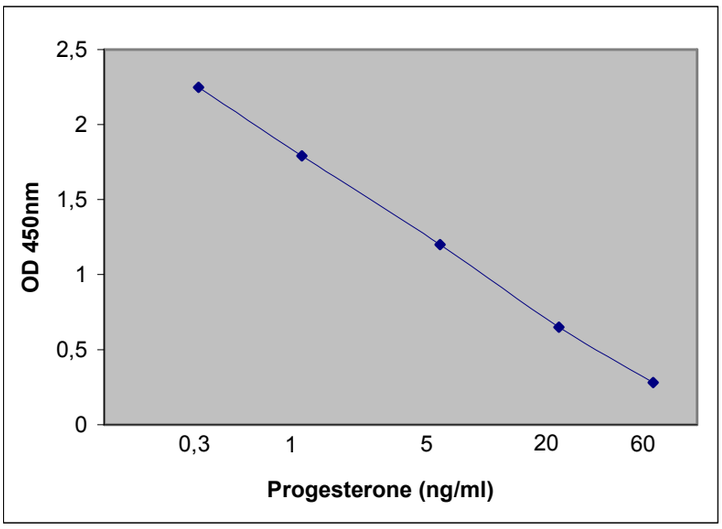
1. Calculate the mean optical density of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter curve is recommended.
3. Calculate the mean optical density of each unknown duplicate.
4. Read the values of the unknowns directly off the calibrator curve.
5. If a sample reads more than 60 ng/ml then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

13. TYPICAL TABULATED DATA

Calibrator	OD 1	OD 2	Mean OD	Value (ng/ml)
A	2.392	2.364	2.378	0
B	2.262	2.231	2.247	0.3
C	1.776	1.805	1.791	1
D	1.208	1.189	1.199	5
E	0.623	0.615	0.619	20
F	0.287	0.272	0.280	60
Unknown	0.725	0.753	0.739	15.4

14. TYPICAL CALIBRATOR CURVE

Sample curve only. **Do not** use to calculate results.



15. PERFORMANCE CHARACTERISTICS

15.1 Sensitivity

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator A (based on 10 replicate analyses) minus 2 SD. Therefore, the sensitivity of the Direct Progesterone ELISA kit is **0.1 ng/ml.**

15.2 Specificity (CROSS REACTIVITY)

The following compounds were tested for cross-reactivity with the Direct Progesterone ELISA kit with progesterone cross-reacting at 100%.

Steroid	%Cross Reactivity
Progesterone	100
11α-OH-Progesterone	100
Deoxycorticosterone	1.7
17-OH-Progesterone	0.4
5α-Androstan-3β, 17β-diol	0.3
Corticosterone	0.3
Pregnenolone	0.2

The following steroids were tested but cross-reacted at less than 0.1%: Cortisol, Cortisone, Danazol, DHEAS, Estradiol, 5β-Pregnan-3α, 17α, 21α-triol-20-one, 5β-Pregnan-3α, 17-diol-20-one, Pregnan-3α, 20α-diol and Testosterone.

15.3 Intra-Assay Precision

Two samples were assayed ten times each on the same calibrator curve. The results (in ng/ml) are tabulated below:

Sample	Mean	SD	CV%
1	1.89	0.20	10.6
2	14.24	1.45	10.2

15.4 Inter-Assay Precision

Two samples were assayed ten times over a period of four weeks. The results (in ng/ml) are tabulated below:

Sample	Mean	SD	CV%
1	2.63	0.33	12.6
2	10.15	1.04	10.2

15.5 Recovery

Spiked samples were prepared by adding defined amounts of progesterone to two patient serum samples (1:1). The results (in ng/ml) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1 Unspiked	20	-	-
+5.0	13	12.5	104
+20.0	16	20	80
+60.0	31	40	78
2 Unspiked	45	-	-
+5.0	31	25	124
+20.0	35	32.5	108
+60.0	48	52.5	91

15.6 Linearity

Two patient serum samples were diluted with calibrator A. The results (in ng/ml) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1	10	-	-
1:2	5.3	5	106
1:4	2.16	2.5	86
1:8	1.38	1.25	110
1:16	0.59	0.63	95
2	20	-	-
1:2	10	9.1	91
1:4	5	4.2	84
1:8	2.5	2.0	81
1:16	1.25	1.1	88

15.7 Comparative Studies

The Direct Progesterone ELISA kit (Kit A) was compared with a competitors coated tube RIA kit (Kit B). The results (in ng/ml) are tabulated below:

Group	Kit A Mean	Kit B Mean
33 Samples	3.05	2.76

16. EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	Mean (ng/ml)	Abs. Range (ng/ml)
Males	0.53	ND-1.35
Females	8.37	ND-70.0
Postmenopausal Females	0.46	ND-4.0

17. REFERENCES

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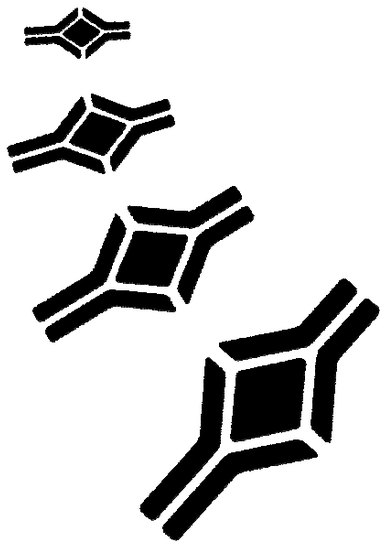

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