Progesterone ELISA

For the direct quantitative determination of Progesterone in human serum.

For In Vitro Diagnostic Use within the United States of America.
This product is for Research Use Only outside of the United States of America.

Catalog Number: 11-PROHU-E01

Size: 96 wells

Version: 8.0 – ALPCO 2.0

INTENDED USE

For the direct quantitative determination of Progesterone by enzyme immunoassay using human serum.

PRINCIPLE OF THE TEST

The Progesterone ELISA is a competitive immunoassay. Competition occurs between an unlabeled antigen (present in calibrators, controls, and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microplate. The washing and decanting procedures remove unbound materials. After the washing step, the TMB substrate is added which reacts with HRP to form a blue-colored product that is inversely proportional to the amount of progesterone present. Following an incubation, the enzymatic reaction is terminated by addition of the stop solution, converting the color from blue to yellow. The absorbance is measured on a microtiter plate reader at 450 nm. A set of calibrators is used to plot a calibration curve from which the amount of progesterone in patient samples and controls can be directly read.

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, super ovulatory drugs, estrogen replacement therapy medication, and GnRH analogues. The removal of ovarian function following surgical oophorectomy or chemotherapy may influence serum progesterone values.

PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is for use by trained laboratory personnel (professional use only). For laboratory in vitro use only.
2. Practice good laboratory practices when handling kit reagents. This includes:
   - Do not pipette by mouth.
   - Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
   - Wear protective clothing and disposable gloves when handling the specimens and kit reagents.
   - Wash hands thoroughly after performing the test.
   - Avoid contact with eyes; use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
3. Users should have a thorough understanding of this protocol for the successful use of this kit.
4. Do not use the kit beyond the expiration date stated on the label.
5. If the kit reagents are visibly damaged, do not use the test kit.
6. 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.
7. All kit reagents and samples should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and samples.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
10. A calibration curve must be established for every run.
11. It is recommended to all customers to prepare their own control materials or serum pools which should be included in every run at the high and low level for assessing the reliability of results.
12. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the controls do not reflect established ranges.
13. When dispensing the substrate and stop solution, do not use pipettes in which these liquids will come into contact with any metal parts.
14. The substrate solution (TMB) is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.

15. Do not use grossly hemolyzed, grossly lipemic, icteric, or improperly stored serum.
16. Samples containing azide or thimerosal are not compatible with this kit, they may lead to false results.
17. Samples values above the measuring range of the kit may be reported as >60 ng/mL. If further dilution and retesting is required, only calibrator A may be used to dilute serum samples. The use of any other reagent may lead to false results.
18. Avoid microbial contamination of reagents.
19. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
20. To prevent the contamination of reagents, do not pour reagents back into the original containers.
21. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.
22. Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
23. This kit contains 1 M sulfuric acid in the stop solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
24. The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
25. Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
26. If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the **REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED** section. Both the type and speed of shaker used can influence the optical densities and test results. If a different type of shaker and/or speed is used, the user is responsible for validating the performance of the kit.
27. Do not reuse the microplate wells, they are for SINGLE USE only.
28. To avoid condensation within the microplate wells in humid environments, do no open the pouch containing the microplate until it has reached room temperature.
29. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

SAFETY CAUTIONS AND WARNINGS

BIOHAZARDS

The reagents should be considered a potential biohazard and handled with the same precautions applied to blood specimens. All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.
The calibrators and controls provided with the kit contain material(s) of human origin that have been tested and found to be negative for the presence of HBsAg and antibodies to HCV, HIV 1/2, and HIV NAT. However, no test method can offer complete assurance that any viable pathogens are absent. Therefore, these components should be considered a potential biohazard and handled with the same precautions as applied to any blood sample, following good laboratory practices.

CHEMICAL HAZARDS

Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB substrate (contains tetramethylbenzidine) and stop solution (contains sulfuric acid). If contacted with any of these reagents, wash with plenty of water and refer to SDS for additional information.

SAMPLE 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 at room temperature and carefully remove the serum layer. Store at 2 – 8 °C for up to 24 hours or at -10°C or lower if the analyses are to be done later. Consider all human samples as possible biohazardous materials and take appropriate precautions when handling.

SAMPLE PRETREATMENT

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

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 25 µL.
2. Calibrated multichannel pipettes to dispense 50 µL, 100 µL, and 150 µL.
3. Calibrated multichannel pipettes to dispense 300 µL (if washing manually).
4. Automatic plate washer (recommended).

5. Microplate shaker:
    a. Orbital Shaker (3 mm diameter) set to 600 rpm or
    b. Reciprocating shaker (1.5” stroke length) set to 180 oscillations/minute.
6. Disposable pipette tips.
7. Distilled or deionized water
8. Calibrated microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater
9. Timer

REAGENTS PROVIDED

1. **MPL Microplate**
   Contents: One anti-progesterone polyclonal antibody-coated 96-well (12x8) microplate in a resealable pouch with desiccant.
   Format: Ready to Use
   Storage: Refrigerate at 2–8°C
   Stability: Unopened: Stable until the expiration date printed on the label.
   After Opening: Stable for four weeks.

2. **HRP CONJ CONC HRP Conjugate Concentrate**
   Contents: One bottle containing Progesterone-Horseradish Peroxidase (HRP) conjugate in a protein-based buffer with a non-mercury preservative.
   Format: Concentrated; Requires Preparation
   Volume: 0.4 mL/bottle
   Storage: 2- 8°C
   Stability: Unopened: Stable until the expiration date printed on the label.
   After Opening: Stable for four weeks.

   X101 DILUTE 1:101 BEFORE USE

Preparation of 1X Working HRP Conjugate:	Dilute 1:101 in assay buffer before use (e.g., 20 µL of conjugate concentrate in 2 mL of assay buffer). If the whole microplate is to be used dilute 120 µL of conjugate concentrate in 12 mL of assay buffer. Discard any that is left over.

3. **CAL A – F Calibrators A-F**
   Contents: Six bottles of calibrator containing specified progesterone concentrations. Human serum-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of progesterone.

   * Listed below are approximate concentrations, please refer to bottle labels for exact Concentrations: 0, 0.3, 1, 5, 20, 60 ng/mL.
   Format: Ready to use.
   Volume: Calibrator A: 2.0 mL/Bottle
   Calibrator B – F: 0.5 mL/Bottle
   Storage: Refrigerate at 2–8°C
   Stability: Unopened: Stable until the expiration date printed on the label.
   After Opening: Stable for four weeks.

4. **CONTROL 1-2 Controls 1-2** — Ready to Use
   Contents: Two bottles containing different progesterone concentrations in a human serum-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of progesterone. Refer to QC certificate for the acceptable range.
   Format: Ready to use.
   Volume: 0.5 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: Unopened: Stable until the expiration date printed on the label.
   After Opening: Stable for four weeks.

5. **WASH BUFF CONC Wash Buffer Concentrate**
   Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
   Format: Concentrated; requires preparation
   Volume: 50 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: Unopened: Stable until the expiration date printed on the label.
   After opening: Stable for four weeks.
   Following preparation: The 1X working wash buffer is stable for 2 weeks following preparation, assuming Good Laboratory Practices are adhered to. To prevent microbial growth, prepare the 1X working wash buffer in a clean container and store under refrigerated conditions (2–8°C) when not in use.

X10 DILUTE 1:10 BEFORE USE

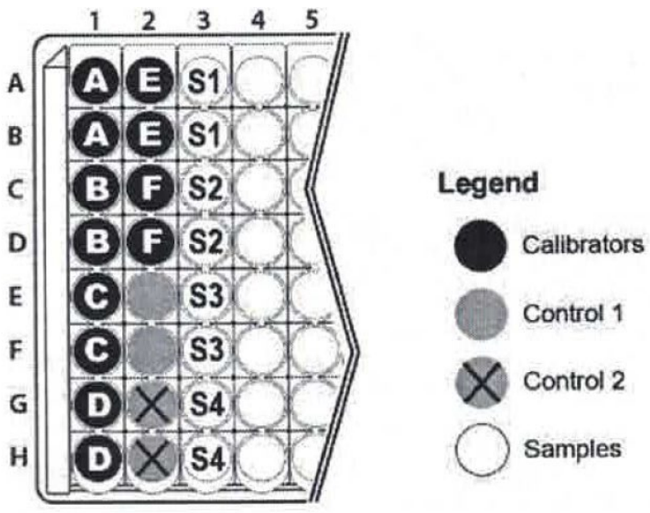
Preparation of 1X Working Wash Buffer:	Dilute 1:10 in distilled or deionized water before use. If the whole microplate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of distilled or deionized water.

6. **ASY BUFF Assay Buffer**
   Contents: One bottle containing a protein-based buffer with a non-mercury preservative.
   Format: Ready to use
   Volume: 15 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: Unopened: Stable until the expiration date printed on the label.
   After Opening: Stable for four weeks.

7. **TMB SUB TMB Substrate** — Ready to Use
   Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
   Format: Ready to use.
   Volume: 16 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: Unopened: Stable until the expiration date printed on the label.
   After Opening: Stable for four weeks.

8. **STOP Stop Solution**
   Contents: One bottle containing 1M sulfuric acid.
   Format: Ready to use.
   Volume: 6 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: Unopened: Stable until the expiration date printed on the label.
   After Opening: Stable for four weeks
   Safety:  Refer to product SDS.
   Warning

RECOMMENDED ASSAY LAYOUT



ASSAY PROCEDURE

Sample Pretreatment: None.

All reagents must reach room temperature before use. Calibrators, controls and samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. After all kit components have reached room temperature, mix gently by inversion.
2. Prepare working solutions of the HRP conjugate and wash buffer. (See **REAGENTS PROVIDED** section).
3. Plan the microplate wells to be used for calibrators, controls, and samples. (See **RECOMMENDED LAYOUT**). Remove strips from the microplate frame that will not be used and place them in the bag with desiccant. Reseal the bag with the unused strips and return it to the refrigerator.

4. Pipette 25 µL of each calibrator, control, and sample into correspondingly labelled wells in duplicate.
5. Pipette 100 µL of the 1X working HRP Conjugate into each well. (The use of a multichannel pipette is recommended.)
6. Incubate on a plate shaker for 60 minutes at room temperature.
7. Wash the microplate wells with an automatic microplate washer (preferred) or manually as stated below.

   Automatic: Using an automatic microplate washer, perform a 3-cycle wash using 300 µL/well of 1X working Wash Buffer (3 x 300 µL). One cycle consists of aspirating all wells then filling each well with 300 µL of 1X working Wash Buffer. After the final wash cycle, aspirate all wells and then tap the microplate firmly against absorbent paper to remove any residual liquid.

   Manual: For manual washing, perform a 3-cycle wash using 300 µL/well of 1X working Wash Buffer (3 x 300 µL). One cycle consists of aspirating all wells by briskly emptying the contents of the wells over a waste container, then pipetting 300 µL of 1X working Wash Buffer into each well using a multichannel pipette. After the final wash cycle, aspirate all wells by briskly emptying the contents over a waste container and then tap the microplate firmly against absorbent paper to remove any residual liquid.

8. Pipette 150 µL of the TMB substrate into each well (the use of a multichannel pipette is recommended).
9. Incubate on a plate shaker for 10-20 minutes at room temperature.
10. Pipette 50 µL of stop solution into each well (use of a multichannel pipette is recommended) at the same timed intervals as in step 8. Gently tap the microplate frame to mix the contents of the wells.
11. Read the plate on a microplate reader at 450 nm within 20 minutes after addition of the stop solution.

CALCULATIONS

1. Calculate the mean optical density of each calibrator, control, and sample duplicate.
2. Use a 4-parameter or 5-parameter curve fit with immunoassay software to generate a calibration curve.
3. The immunoassay software will calculate the concentrations of the controls and samples using mean optical density values and the calibration curve.
4. If a sample reads more than 60 ng/mL and needs to be diluted and retested, then dilute with Calibrator A not more than 1:8. The result obtained must be multiplied by the dilution factor.

QUALITY CONTROL

When assessing the validity of the test results, the following criteria should be evaluated:

1. The calibrator with the highest concentration meets the % binding acceptable range as stated in the QC Certificate.

   %Binding = (OD of calibrator/OD of Calibrator A) X 100
2. The values obtained for the kit controls are within the acceptable ranges stated in the QC certificate.
3. The results of any external controls that were used meet the acceptable ranges.

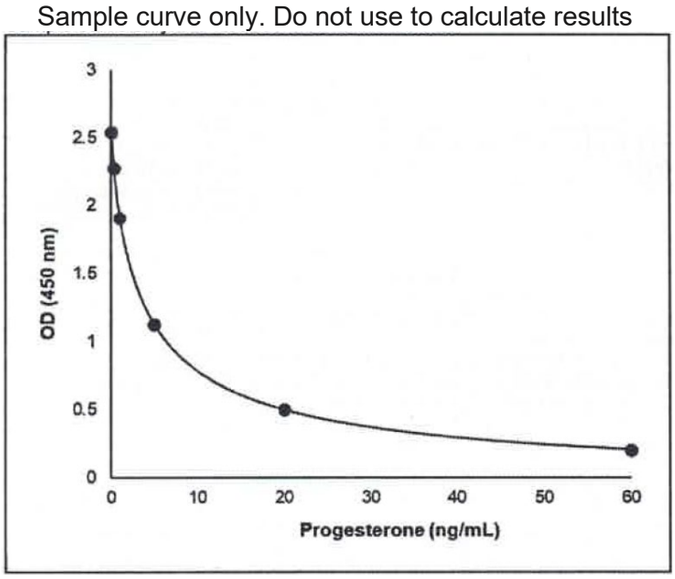
TYPICAL TABULATED DATA

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

Calibrator	Mean OD (450 nm)	% Binding	Value (ng/mL)
A	2.538	100	0
B	2.274	90	0.3
C	1.909	75	1
D	1.124	44	5
E	0.502	20	20
F	0.200	8	60
Unknown	0.853	-	8.6

TYPICAL CALIBRATOR CURVE

Sample curve only. Do not use to calculate results



PERFORMANCE CHARACTERISTICS

SENSITIVITY

The lower detection limit is calculated from the calibration 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 Progesterone ELISA kit is **0.1 ng/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity in the 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-Androstane-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.

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

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

RECOVERY

Spiked samples were prepared by adding defined amounts of progesterone to two 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

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

COMPARATIVE STUDIES

The 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

REFERENCE RANGES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values. (ND = Not detectable)

Group	Mean (ng/mL)	Range (ng/mL)
Males	0.53	ND–1.35
Females	8.37	ND–70.0
Postmenopausal Females	0.46	ND–4.0

REFERENCES

1. Grodsky G, et al., eds. Review of Physiological Chemistry, 17th edition. Los Altos, CA: Lange Medical Publications; 1979:511.
2. Abraham GE, et al. Simultaneous Radioimmunoassay of Plasma FSH, LH, Progesterone, 17-hydroxyprogesterone, and Estradiol-17 beta During the Menstrual Cycle. *J Clin Endocrinol Metab*. 1972; 34(2):312–8.
3. Ryan K, Greep R, Astwood E, eds. Handbook of Physiology, Vol. II. American Physiology Society, 1973:285.
4. Israel R, et al. Single Luteal Phase Serum Progesterone Assay as an Indicator of Ovulation. *Am J Obstet Gynecol*. 1972; 112(8):1043–6.
5. Soules MR, et al. The Function of the Corpus Luteum of Pregnancy in Ovulatory Dysfunction and Luteal Phase Deficiency. *Fertil Steril*. 1981; 36(1):31–6.
6. Manganiello PD, et al. Serum Progesterone, 17 Alpha-Hydroxyprogesterone, Human Chorionic Gonadotropin, and Prolactin in Early Pregnancy and a Case of Spontaneous Abortion. *Fertil Steril*. 1981; 36(1):55–60.
7. Matthews CP, et al. Serum Progesterone Levels as an Aid in the Diagnosis of Ectopic Pregnancy. *Obstet Gynecol*. 1986; 68(3):390–4.
8. Check JH, et al. Falsely Elevated Steroidal Assay Levels Related to Heterophile Antibodies Against Various Animal Species. *Gynecol Obstet Invest*. 1995; 40(2):139–40.