# Progesterone Test System
# Product Code: 4825-300

## 1.0 INTRODUCTION

**Intended Use: The Quantitative Determination of Progesterone Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay**

## 2.0 SUMMARY AND EXPLANATION OF THE TEST

Measurement of progesterone in serum or plasma is considered to be the most reliable way to assess its rate of production.

Progesterone is a steroid hormone, which plays an important role in the preparation for and maintenance of pregnancy. It is synthesized from cholesterol via pregnenolone — then rapidly metabolized to pregnanediol primarily in the liver.[2, 9,13] The ovary and placenta are the major production sites; but a small amount is also produced by the adrenal cortex in both men and women. Circulating progesterone levels, which are characteristically low during the follicular phase, increase sharply during the luteal phase of menstrual cycles, reaching a maximum approximately 5 to 10 days after the midcycle LH peak.[12] Unless pregnancy occurs, a steep decline to follicular levels sets in about 4 days before the next menstrual period. This pattern constitutes the rationale behind the well established use of serum progesterone measurements as a simple and reliable method for ovulation detection.[3,4,16]

For routine measurements, immunoassays using steroid specific antibodies are preferred. Initial immunoassays for serum progesterone used organic solvents to remove the steroid from endogenous binding proteins such as corticosteroid binding globulin (CBG) and albumin. Direct measurement of progesterone in serum or plasma is considered to be the method of choice for routine applications. Both RIA and EIA (and some FIA) are available in the market. Since RIA involves handling radioactivity and causes radioactive waste disposal issues, various non-isotopic methods have replaced the RIA. These methods use very specific antibodies to determine levels of progesterone in circulation.

The Progesterone ELISA kit uses a specific anti-progesterone antibody, and does not require sample extraction of serum or plasma. Cross-reactivity to other naturally occurring and structurally related steroids is low.

The employment of several serum references of known progesterone concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with progesterone concentration.

## 3.0 PRINCIPLE

**Competitive Enzyme Immunoassay (TYPE 7):**
The essential reagents required for a enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen.

Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. The interaction is illustrated by the followed equation:

$$^{Enz}Ag + Ag + Ab_{Btn} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{Btn} + {}^{Enz}AgAb_{Btn}$$

$Ab_{Btn}$ = Biotinylated Antibody (Constant Quantity)

Ag = Native Antigen (Variable Quantity)

$^{Enz}Ag$ = Enzyme-antigen Conjugate (Constant Quantity)

$AgAb_{Btn}$ = Antigen-Antibody Complex

$^{Enz}Ag\ Ab_{Btn}$ = Enzyme-antigen Conjugate -Antibody Complex

$k_a$ = Rate Constant of Association

$k_{-a}$ = Rate Constant of Disassociation

$K = k_a / k_{-a}$ = Equilibrium Constant

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.

$AgAb_{Btn} + {}^{Enz}AgAb_{Btn} + \underline{Streptavidin_{cw}} \Rightarrow \underline{immobilized\ complex}$

$\underline{Streptavidin_{cw}}$ = Streptavidin immobilized on well

$\underline{Immobilized\ complex}$ = sandwich complex bound to the solid surface

The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

## 4.0 REAGENTS

**Materials Provided:**

**A. Progesterone Calibrators – 1ml/vial - Icons A-G**
Seven (7) vials of serum reference for progesterone at concentrations of 0 (**A**), 0.3 (**B**), 2.0 (**C**), 5.0 (**D**), 15 (**E**), 30 (**F**) and 60.0 (**G**) ng/ml. Store at 2-8°C. A preservative has been added. The calibrators can be expressed in molar concentrations (nM/L) by multiplying by 3.18. For example: 1ng/ml x 3.18 = 3.18 nM/L

**B. Progesterone Enzyme Reagent – 6.0 ml/vial**
One (1) vial of Progesterone (Analog)-horseradish peroxides (HRP) conjugate in a protein stabilizing matrix with red dye. Store at 2-8°C.

**C. Progesterone Biotin Reagent – 6.0 ml - Icon**
One (1) bottle of reagent contains anti-Progesterone biotinylated purified rabbit IgG conjugate in buffer, yellow dye and preservative. Store at 2-8°C.

**D. Streptavidin Coated Plate – 96 wells –Icon**
One 96-well microplate coated with 1.0 µg/ml streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

**E. Wash Solution Concentrate – 20ml – Icon**
One (1) vial contains a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

**F. Substrate Reagent – 12ml/vial - Icon S^N**
One (1) bottle contains tetramethylbenzidine (TMB) and hydrogen peroxide ($H_2O_2$) in buffer. Store at 2-8°C.

**G. Stop Solution – 8ml/vial - Icon**
One (1) vial contains a strong acid ($H_2SO_4$). Store at 2-30°C.

**H. Product Instructions**

**Note 1:** Do not use reagents beyond the kit expiration date.
**Note 2:** Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
**Note 3:** Above reagents are for a single 96-well microplate.

**4.1 Required But Not Provided:**
1. Pipette capable of delivering 25 µl and 50 µl with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.350ml volumes with a precision of better than 1.5%.
3. Adjustable volume (200-1000µl) dispenser(s) for conjugate.
4. Microplate washer or a squeeze bottle (optional).
5. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
6 Absorbent Paper for blotting the microplate wells.
7 Plastic wrap or microplate cover for incubation steps.
8 Vacuum aspirator (optional) for wash steps.
9 Timer.
10 Quality control materials.

## 5.0 PRECAUTIONS

**For In Vitro Diagnostic Use**
**Not for Internal or External Use in Humans or Animals**

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

**Safe Disposal of kit components must be according to local regulatory and statutory requirement.**

## 6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum or heparanised plasma in type, and taken with the usual precautions in the collection of venipuncture samples. For accurate comparison to establish normal values, a fasting morning serum sample should be obtained. The blood should be collected in a redtop (with or without gel additives) venipuncture tube or for plasma use evacuated tube(s) containing heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml of the specimen is required.

## 7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

## 8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.

***Note1 : Do not use the substrate if it looks blue.***
***Note 2: Do not use reagents that are contaminated or have bacteria growth.***

## 9.0 TEST PROCEDURE

*Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 27°C).*
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025 ml (25 µL) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.050 ml (50µl) of Progesterone Enzyme Reagent to all wells.
4. Swirl the microplate gently for 10-20 seconds to mix.
5. Add 0.050 ml (50µl) Progesterone Biotin Reagent to all wells.
6. Swirl the microplate gently for 10-20 seconds to mix.
7. Cover and incubate for 60 minutes at room temperature.
8. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
9. Add 350µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
10. Add 0.100 ml (100µl) of Substrate solution to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**
**DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION**
11. Incubate at room temperature for twenty (20) minutes.
12. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
13. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm. **The results should be read within thirty (30) minutes of adding the stop solution.**

**Note**: Dilute the samples suspected of concentrations higher than 60ng/ml 1:5 and 1:10 with progesterone '0' ng/ml calibrator or male patient serum pools with a known low value for progesterone.

## 10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of progesterone in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding progesterone concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of progesterone for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.517) intersects the dose response curve at (08.1ng/ml) progesterone concentration (See Figure 1).

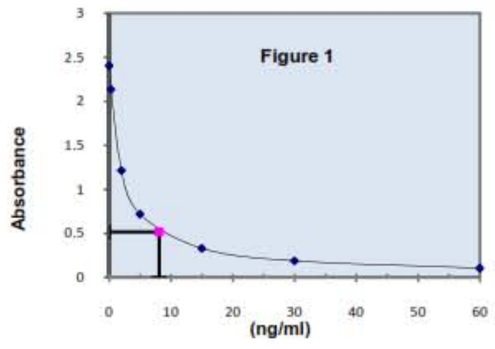
**Note:** Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

**EXAMPLE 1**

| Sample I.D. | Well Number | Abs (A) | Mean Abs (B) | Value (ng/ml) |
|---|---|---|---|---|
| Cal A | A1 | 2.420 | 2.406 | 0 |
| | B1 | 2.391 | | |
| Cal B | C1 | 2.155 | 2.137 | 0.3 |
| | D1 | 2.119 | | |
| Cal C | E1 | 1.248 | 1.215 | 2.0 |
| | F1 | 1.183 | | |
| Cal D | G1 | 0.721 | 0.719 | 5.0 |
| | H1 | 0.717 | | |
| Cal E | A2 | 0.338 | 0.330 | 15.0 |
| | B2 | 0.322 | | |
| Cal F | C2 | 0.187 | 0.188 | 30.0 |
| | D2 | 0.190 | | |
| Cal G | G2 | 0.107 | 0.105 | 60.0 |
| | H2 | 0.104 | | |
| Pat# 1 | A3 | 0.525 | 0.517 | **8.1** |
| | B3 | 0.510 | | |

*The above data and table below is for example only. Do not use it for calculating your results.

**Figure 1**




## 11.0 Q.C. PARAMETERS

**In order for the assay results to be considered valid the following criteria should be met:**
1. The absorbance (OD) of calibrator 0 ng/ml should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

## 12.0 RISK ANALYSIS

**12.1 Assay Performance**
1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
10. Patient specimens with Progesterone levels higher than 60ng/ml may be diluted (1:5 or 1:10) with progesterone '0 ng/ml' calibrator or male patient serum pools with a known low value for progesterone.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from info@gentaur.com

**12.2 Interpretation**
1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted,
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

## 13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a "normal" adult population and females during gestation the expected ranges for the Progesterone AccuBind™ ELISA Test System are detailed in Table 1. During pregnancy the progesterone serum levels rise rapidly till the end of third trimester[17].

**TABLE I**
**Expected Values for the Progesterone Test System**

| | (ng/ml) | (nmol/L) |
|---|---|---|
| Prepubertal Child (1-10 yr) | 0.07 – 0.52 | 0.2-1.7 |
| Adult man | 0.13 – 1.22 | 0.4 – 3.88 |
| ***Adult woman*** | | |
| Follicular phase | 0.15 – 1.40 | 0.5 – 4.4 |
| Luteal phase | 2.0 – 25.0 | 6.4 – 79.5 |
| ***Pregnant woman*** | | |
| First trimester | 7.25 – 90.0 | 23 – 286 |
| Second trimester | 19.5 – 91.0 | 62 – 289 |
| Third trimester | 49.0 – 422.0 | 153 – 1342 |
| ***Postmenopausal woman*** | 0.0 – 0.80 | 0.0 – 2.55 |

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal" persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

## 14.0 PERFORMANCE CHARACTERISTICS

**14.1 Precision**
The within and between assay precision of the progesterone AccuBind™ Microplate EIA Test System were determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

**TABLE 2**
Within Assay Precision (Values in ng/ml )

| Sample | N | X | σ | C.V. |
|---|---|---|---|---|
| Low | 20 | 1.0 | 0.093 | 9.3% |
| Normal | 20 | 11.1 | 0.344 | 3.1% |
| High | 20 | 40.5 | 1.155 | 2.9% |

**TABLE 3**
Between Assay Precision (Values in ng/ml )

| Sample | N | X | σ | C.V. |
|---|---|---|---|---|
| Low | 10 | 1.1 | 0.10 | 9.9% |
| Normal | 10 | 10.8 | 0.76 | 7.0% |
| High | 10 | 39.2 | 2.18 | 5.6% |

*As measured in ten experiments in duplicate over a ten day period.

**14.2 Sensitivity**
The progesterone AccuBind™ Microplate EIA Test System has a sensitivity of 0.105 ng/ml. The sensitivity was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

**14.3 Accuracy**
The Progesterone AccuBind™ Microplate ELISA Test System was compared with a chemiluminescence immunoassay method. Biological specimens from low, normal and high progesterone level populations were used (The values ranged from < 0.15 ng/ml – 128 ng/ml). The total number of such specimens was 60. The least square regression equation and the correlation coefficient were computed for this method in comparison with the reference method. The data obtained is displayed in Table 4.

**TABLE 4**

| Method | Mean (x) | Least Square Regression Analysis | Correlation Coefficient |
|---|---|---|---|
| **This** | 14.59 | y= -1.223+1.018(x) | 0.989 |
| **Method (y)** | | | |
| **Reference (X)** | 15.53 | | |

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

**14.4 Specificity**
The % cross reactivity of the progesterone antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of progesterone needed to displace the same amount of labeled analog.

| Substance | Cross Reactivity |
|---|---|
| Progesterone | 100.000 |
| 17OH-Progesterone | 0.375 |
| Androstenedione | 0.158 |
| Cortisone | 0.014 |
| Corticosterone | 0.347 |
| Cortisol | 0.005 |
| Danazol | 0.003 |
| Dihydotestosterone | 0.006 |
| DHEA sulfate | 0.002 |
| Estradiol | 0.004 |
| Estrone | 0.003 |
| Estriol | 0.002 |
| Prednisone | 0.023 |
| Testosterone | 0.015 |

## 15.0 REFERENCES


1. Abraham GE. The application of natural steroid radioimmunoassay to gynecologic endocrinology. In: Abraham GE, editor. Radioassay Systems in Clinical Endocrinology, Basel: Marcel Dekker,: 475-529 (1981).
2. Aufrere MB, Benson H. Progesterone: an overview and recent advances. , **65**:783-800 (1976).
3. Bauman J, "Basal body temperature: unreliable method of ovulation detection", *Fertility Sterility*, **36**:**729**-33, (1981).
4. Brown JB," Timing of ovulation", *Med J Austral*, **2**:780-3 (1977).
5. Gautray JP, et al, "Clinical investigation of the menstrual cycle: clinical, endometrial and endocrine aspects of luteal defects",. *Fertility Sterility*, **35**:296-303 (1981).
6. Hensleigh PA, Fainstat T, "Corpus luteum dysfunction: serum progesterone levels in diagnosis and assessment of therapy for recurrent and threatened abortion", *Fertility Sterility*, **32**:396-9. (1979).
7. Hernandez JL, et al," Direct evidence of luteal insufficiency in women with habitual abortion", *Obstetric Gynecology* , 49:705-8.( 1977).
8. Jones G. Luteal phase defects. In: Behrman SJ, Kistner RW, editors. Progress in Infertility. Boston: Little, Brown and Company, 2nd ed., 1975: 299-324.
9. Klopper A, Fuchs F. Progestagens. In: Fuchs F, Klopper A, editors. Endocrinology of Pregnancy. Hagerstown: Harper & Row,: 99-122 (1977).
10. Lehmann F, Bettendorf G," The endocrine shift from a normal cycle to anovulation",: Insler V, Bettendorf G, editors. Advances in Diagnosis and Treatment of Infertility. Amsterdam: Elseveir/North Holland, 105 - 13 (1981).
11. March CM. Luteal phase defects. In: Mishell DR, Davajan V, editors. Reproductive Endocrinology, Infertility and Contraception. Philadelphia: F. A. Davis Company, 469-76, (1979).
12. March CM, Goebelsmann U, Nakamura RM, Mishell Dr. Roles of estradiol and progesterone in eliciting the midcycle luteinizing hormone and follicle stimulating hormone surges. *J Clin Endocrinol Metab*, **49**:507-13 (1979).
13. BIO-ED slide/seminar educational program, Rochester: Bioeducational Publications (1981).
14. Radwanska E, et al, "Plasma progesterone and estradiol estimations in the diagnosis and treatment of luteal insufficiency in menstruating infertile women", *Acta Eur Fertility*, **7**:39-47(1976).
15. Radwanska E, et al,"Plasma progesterone levels in normal and abnormal early human pregnancy", *Fertility Sterility* **30**:398-402 (1978).
16. Radwanska E, et al, "Single midluteal progesterone assay in the management of ovulatory infertility",. *J Reprod Med*, **26**:85-9 (1981).
17. Tietz, Reference Information for the Clinical Laboratory. In *Textbook of Clinical Chemistry*, 3rd Ed Burtis, C.A., Ashwood, R.A. W.B. Saunders: Philadelphia, 1999; 1831.



**Revision: 5** **Date: 030912** **DCO: 0638**


| Size | | 96(A) | 192(B) |
|---|---|---|---|
| Reagent (fill) | A) | 1ml set | 1ml set |
| | B) | 1 (6ml) | 2 (6ml) |
| | C) | 1 (6ml) | 2 (6ml) |
| | D) | 1 plate | 2 plates |
| | E) | 1 (20ml) | 1 (20ml) |
| | F) | 1 (12ml) | 2 (12ml) |
| | G) | 1 (8ml) | 2 (8ml) |

For Orders and Inquiries, please contact


**Gentaur Molecular Products**
**Voortstraat 49**
**1910 Kampenhout, Belgium**
**http://www.gentaur-worldwide.com**