REFERENCES

1. Radwanska, E., Frankenberg, J., and Allen, E., Plasma progesterone levels in normal and abnormal early human pregnancy. *Fertility and Sterility*, 1978; 30, 398-402.
2. Autrere,M.B., and Benson, H., Progesterone: An overview and recent advances, *J. Par. Sci.*, 1976; 65: 783-800.
3. March, C.M., Goebelsmann, U., Nakamura, R.M., and Mishell, D.R. Jr., Roles of estradiol and progesterone in eliciting the midcycle luteinizing hormone and follicle-stimulating hormone surges, *J. Clin. Endo. Metab.,* 1979; 49, 507-513.
4. Ross, G.T., Vande Wiele, R.L., and Frantz, A.G., The Ovaries and the breasts. In: Williams, R.H., ed., *Textbook of Endocrinology*. Saunders Company, Philadelphia; 1981: 355-411.
5. Chattoraj, S.C., Endocrine function. In: Tietz, N.W., ed., *Fundamentals of Clinical Chemistry*. Saunders Company, Philadelphia; 1976: 699-823.
6. Shepard, M.K., and Senturia, Y.D., Comparison of serum progesterone and endometrial biopsy for confirmation of ovulation and evaluation of luteal function. *Fertility and Sterility*, 1977; 28: 541-548.
7. Johansson, E.D.B., and Jonasson, L.-E., Progesterone levels in amniotic fluid and plasma from women: I. Levels during normal pregnancy. *Acta Obstet. Gynec. Scand.*, 1971; 50: 339-343.
8. USA Center for Disease Control/National Institute of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories"" 1984.
9. Tietz, N.W. ed., *Clinical Guide to Laboratory Tests*, 3rd Edition, W.B. Saunders, Co., Philadelphia, 1995: 509-512.
10. *ICN Guide to Endocrine Testing*. Diagnostic Division, ICN Biomedicals, Inc. pp. 2:20-27.

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Progesterone ELISA

Catalog No. PG129S (96 Tests)

INTENDED USE

The Progesterone ELISA kit is used for the quantitative measurement of Progesterone in human serum or plasma. For research use only.

SUMMARY AND EXPLANATION

Progesterone is a C21 steroid which is synthesized from both tissue and circulating cholesterol. Cholesterol is transformed to Progesterone which is then converted via a combined dehydrogenase and isomerase to progesterone. The principle production sites are the adrenals and ovaries and the placenta during pregnancy. The majority of this steroid is metabolized in the liver to pregnanediol and conjugated as a glucuronide prior to excretion by the kidneys. Progesterone exhibits a wide variety of end organ effects. The primary role of progesterone is exhibited by the reproductive organs. In males, progesterone is a necessary intermediate for the production of corticosteroids and androgens. In females, progesterone remains relatively constant throughout the follicular phase of the menstrual cycle. The concentration then increases rapidly following ovulation and remains elevated for 4-6 days and decreases to the initial level 24 hours before the onset of menstruation. In pregnancy, placental progesterone production rises steadily to levels of 10 to 20 times those of the luteal phase peak. Progesterone measurements are thus performed to determine ovulation as well as to characterize luteal phase defects. Monitoring of progesterone therapy and early stage pregnancy evaluations comprise the remaining uses of progesterone assays. Progesterone EIA kits are designed for the measurement of total progesterone in human serum or plasma.

PRINCIPLE OF THE TEST

The CBI Progesterone is a solid phase competitive ELISA. The samples and Progesterone enzyme conjugate are added to the wells coated with anti-Progesterone monoclonal antibody. Progesterone in the patient's sample competes with a Progesterone enzyme conjugate for binding sites. Unbound Progesterone and Progesterone enzyme conjugate is washed off by washing buffer. Upon the addition of the substrate, the intensity of color is inversely proportional to the concentration of Progesterone in the samples. A standard curve is prepared relating color intensity to the concentration of the Progesterone.

MATERIALS PROVIDED	96 Tests
1. Microwell coated with Progesterone MAb	12x8x1
2. Progesterone Standard set:6 vials (ready to use)	0.25 ml
3. Enzyme Conjugate (20X)	1.2 ml
4. Assay Diluent	24 ml
5. TMB Substrate: 1 bottle (ready to use)	12 ml
6. Stop Solution: 1 bottle (ready to use)	12 ml
7. Wash concentrate (20X): 1 bottle	25 ml

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY

1. Store the kit at 2 – 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun, or strong light.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials:
   The calibrator contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.
2. This kit is designed for research use only.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. It is recommended that standards, control and serum samples be run in duplicate.
7. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION HANDLING

1. It is recommended to collect serum samples with commercially available equipments. The serum samples should be completely colorless even the slight red color shows blood contamination.
2. Specimens may be stored refrigerated at (2-8° C) for 1 days. Store frozen at (-20° C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen serum samples should be completely thawed and mixed well.

REAGENTS PREPARATION

1. Working Reagent A Progesterone-enzyme Conjugate Solution
   Dilute the Progesterone enzyme conjugate 1:21 with assay diluent in a suitable container. For example, dilute 100µl of conjugate with 2ml of assay diluent buffer for 10 wells (A slight excess of solution is made).
2. Wash Buffer
   Prepare 1X Wash Buffer by adding the contents of the bottle (25ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature.

ASSAY PROCEDURE

Prior to assay, bring all reagents to room temperature. Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder
2. Pipet 10 µl of Progesterone standards, control and patient's serum samples.
3. Add 200µl of Progesterone Enzyme Conjugate to all wells.
4. Incubate for 60 minutes at room temperature (18-26° C).
5. Remove liquid from all wells. Wash wells three times with 300 ml of 1X wash buffer. Blot on absorbent paper towels.
6. Add 100 µl of TMB substrate to all wells.
7. Incubate for 15 minutes at room temperature.
8. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stop solution.

CALCULATION OF RESULTS

The standard curve is constructed as follows:

1. Check Progesterone standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.
2. To construct the standard curve, plot the absorbance for Progesterone standards (vertical axis) versus Progesterone standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Standard Curve

Standard	Optical Units (450 nm)
Standard 1 (0 ng/ml)	2.20
Standard 2 (2.5 ng/ml)	1.32
Standard 3 (5 ng/ml)	0.92
Standard 4 (10 ng/ml)	0.65
Standard 5 (20 ng/ml)	0.42
Standard 6 (40 ng/ml)	0.23

EXPECTED VALUES

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values for Progesterone were established by the CBI and may be used as initial guideline ranges only:

Classification	ng/ml
AM-PM	<50

LIMITATIONS OF THE TEST

1. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.