3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Progesterone ELISA

Catalog Number **SE120102**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Progesterone is a C21 steroid which is synthesized from both tissue and circulating cholesterol. 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, 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 10–20 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.

The Progesterone ELISA kit 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 sample competes with a progesterone enzyme conjugate for binding sites. Unbound progesterone and progesterone enzyme conjugate are 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.

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

Components

Materials Provided	96 Tests
Microwell coated with Progesterone MAb	12 x 8 x 1
Progesterone Standard set: 6 vials (ready to use)	0.25 mL
Enzyme Conjugate (20x)	1.2 mL
Assay Diluent	24 mL
TMB Substrate: 1 bottle (ready to use)	12 mL
Stop Solution: 1 bottle (ready to use)	12 mL
Wash concentrate (20x): 1 bottle	25 mL

Reagents and Equipment Required but Not Provided.

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

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

1. It is recommended to collect serum samples with commercially available equipment. 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 day. 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.
   Note: Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

Working Progesterone-enzyme Conjugate Solution (Reagent A)
Dilute the Progesterone enzyme conjugate 21-fold with assay diluent in a suitable container. For example, dilute 100 µL of enzyme conjugate with 2 mL of assay diluent buffer for 10 wells (A slight excess of solution is made).

20× Wash Buffer Concentrate
Prepare 1× wash buffer by adding the contents of the bottle to 475 ml of distilled water. Store 1× wash buffer at room temperature.

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

Procedure
Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

It is recommended that standards, control, and serum samples be run in duplicate.

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.

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. Pipette 10 µL of Progesterone standards, control, and serum samples.
3. Add 200 µL 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 µL 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.

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 the value is checked on every kit.
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.

   Example Of Typical Standard Curve
   The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

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

3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

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 and may be used as initial guideline ranges only:

Classification	ng/mL
AM-PM	<50

Note: The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings, and other diagnostic procedures.

Product profile

Correlation with a Reference ELISA kit

A total of 86 samples were tested by Progesterone ELISA and a reference ELISA kit. Results were as follows:

Correlation	Slope	Intercept
0.94	1.1	0.8

Precision:

Intra-Assay

Precision was determined by assaying 10 replicates of each of three samples; low, normal, and high.

Sample	No. of Replicates	Mean ng/mL	Standard Deviation	Coefficient of Variation (%)
1	10	1.72	0.092	5.36
2	10	10.1	0.16	1.62
3	10	19.7	0.4	2.04

Inter assay

Precision was determined by assaying duplicates of three samples pools in 10 separate runs, using a standard curve constructed for each run.

Sample	No. of Runs	Mean ng/mL	Standard Deviation	Coefficient of Variation (%)
1	10	2.16	0.21	9.68
2	10	9.73	0.85	8.8
3	10	19.1	1.2	6.3

Sensitivity

The sensitivity was determined by calculating the mean plus 2 SD of the standard zero point tested 20 times in the same run.

Sample	No.of Replicates	Mean ng/mL	Standard Deviation	Mean + 2 SD (Sensitivity)
Zero Standard	20	0.04	0.09	0.22 ng/mL

Specificity

The following materials have been checked for cross-reactivity. The percentage indicates cross reactivity at 50% displacement compared to progesterone. Data on the cross-reactivity for several endogenous and pharmaceutical steroids are summarized in the following table:

$$\text{Cross-reactivity (\%)} = \frac{\text{Observed Progesterone Concentration}}{\text{Steroid Concentration}} \times 100$$

Steroid	**Cross-Reactivity**
Progesterone	100%
Androsterone	0.086%
Corticosterone	0.74%
Cortisone	0.11%
Cholesterol	<0.08%
Estradiol	<0.01%
Estrone	0.08%
Estriol	<0.024%
Prednisolone	0.075%
Testosterone	0.1%

Recovery

Known quantities of progesterone were added to a serum that contained a low concentration of progesterone**.**

Expected Value (ng/mL)	**Recovered (ng/mL)**	**Percentage of Recovery**
2.5	2.51	100.4
10	9.16	91.6
20	19.1	95.5

References

1. Radwanska, E. et al., 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. et al., 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. et al., The Ovaries and the breasts. in: Textbook of Endocrinology, Williams, R.H., ed., Saunders Company, (Philadelphia, PA: 1981), 355-411.
5. Chattoraj, S.C., Endocrine function. in: Fundamentals of Clinical Chemistry, Tietz, N.W., ed., Saunders Company, (Philadelphia, PA: 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, Saunders Company, (Philadelphia, PA: 1995), 509-512.
10. ICN Guide to Endocrine Testing, Diagnostic Division, ICN Biomedicals, Inc. pp. 2:20-27.

AI,CH,MAM 10/14-1