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Progesterone (human) ELISA Kit

rev 07/17

(Catalog # K7414-100, 100 assays; Store at 2-8^{0}C)

I. Introduction:

Progesterone is a C21 steroid which is synthesized from both tissue and circulating cholesterol. 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. BioVision's Progesterone kit is a solid phase competitive ELISA Kit. 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 is washed off by wash 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.

II. Application:

Quantitative protein detection, establishing normal range etc.

III. Specificity:

Human Progesterone

IV. Sample Type:

- Serum or Plasma

V. Kit Contents:

Components	K7414-100	Part No.
Microplate coated with Progesterone	12 strips x 8 wells	K7414-100-1
Progesterone Standard: (ready to use)	0.25 ml x 6 vials	K7414-100-2
Assay Diluent	12 ml	K7414-100-3
Enzyme Conjugate (20X)	0.7 ml	K7414-100-4
Progesterone Biotin Conjugate	7 ml	K7414-100-5
Wash Concentrate (20X)	25 ml	K7414-100-6
TMB Substrate (ready to use)	12 ml	K7414-100-7
Stop Solution (ready to use)	12 ml	K7414-100-8

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips.

VII. Storage Conditions and Reagent Preparation:

Store kit at 2-8^{o}C. Keep microwells sealed in a dry bag with desiccants. Spin tubes briefly to bring down all components to the bottom of tubes. Reagents are stable until the expiration of the kit. Do not expose reagent to heat, sun, or strong light. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

- **Wash Concentrate:** Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26° C).
- **Progesterone-Enzyme Conjugate Solution:** Dilute the Progesterone enzyme conjugate 1:21 with assay diluent in a suitable container. For example, dilute 100 µl of conjugate with 2 ml of assay diluent buffer for 10 wells (A slight excess of solution is made). We recommend preparing diluted solution just before use. Diluted solution can be stored at 2-8°C & used within 24 hrs for maximum performance of the assay.

VIII. Warning & Precautions:

- Potential biohazardous materials: The calibrator & controls contains 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.
- This test kit is USA FDA exempt product.
- Do not pipette by mouth.
- 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.

IX. Sample Preparation and Storage:

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. Specimens may be stored refrigerated at (2-8°C) for 1 day. Store frozen at (-20°C) for up to one month. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well.

X. Assay Protocol:

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

1. Place the desired no. of coated strips into the holder. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipet 20 µl of Progesterone Standard, control, and samples into designated wells.
3. Add 100 µl of diluted Progesterone enzyme conjugate to all wells. Shake gently for 20-30 sec. to mix.
4. Add 50 µl of Progesterone Biotin Conjugate to all wells.
5. Cover the plate and incubate for 60 min. at room temperature (18-26°C).
6. Remove liquid from all wells & wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
7. Add 100 µl of TMB substrate to all wells & incubate for 15 min. 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 min. after adding the stop solution.

XI. Calculation: Construct the standard curve; plot the absorbance for the Progesterone standards (vertical axis) versus Progesterone standard concentrations (horizontal axis). Draw the best curve through the points. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Example of a Standard Curve:

Standard	OD (450 nm)
Standard 1 (0 ng/ml)	2.64
Standard 2 (1 ng/ml)	2.06
Standard 3 (5 ng/ml)	1.16
Standard 4 (15 ng/ml)	0.61
Standard 5 (30 ng/ml)	0.30
Standard 6 (60 ng/ml)	0.19

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 BioVision and may be used as initial guideline ranges only

Classification	ng/ml
AM-PM	<50

Performance Characteristics:

1. Correlation with a reference ELISA Kit: A total of 86 samples were tested by BioVision Progesterone ELISA & a reference ELISA kit. Results are as follows:

Correlation	Slope	Intercept
0.94	1.1	0.8

2. 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 sample 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

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

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

4. **Cross-reactivity:** Progesterone:100%, Androsterone: 0.086%, Corticosterone: 0.74%, Cortisone: 0.11%, Cholesterol: <0.08%, Estradiol: <0.01%, Estrone: <0.08%, Estriol: <0.24%, Prednisolone: 0.075%, Testosterone: 0.1%. The percentage indicates cross reactivity 50% displacement compared to Progesterone.

Cross reactivity (%) = Observed Progesterone concentration/steroid concentration X100

XII. RELATED PRODUCTS:

Progesterone (mouse/rat) ELISA Kit (4715)
Progesterone (human) ELISA Kit for Saliva (K7416)
Testosterone (human) ELISA Kit (K7417)
Testosterone (mouse/rat) ELISA Kit (K7418)
Androgen Receptor Antibody (6710)
Androgen Receptor Antibody (Clone 549CT16.1.4) (6711)

FOR RESEARCH USE ONLY! Not to be used on humans.