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Progesterone ELISA Kit

Name	Progesterone ELISA Kit
INTENDED USE	The Progesterone ELISA kit is used for the quantitative measurement of Progesterone in human serum or plasma.
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 progesterone kit is a solid phase competitive ELISA. The samples, working progesterone-enzyme (HRP)

Conjugate and anti-progesterone-Biotin reagent are added to the wells coated with Streptavidin. Progesterone in the patient's specimen competes with a progesterone HRP conjugate for binding sites. Unbound Progesterone and Progesterone enzyme conjugate is washed off by washing buffer. Upon the addition of the TMB 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
Microwells coated with Streptavidin	12x8x1
Progesterone Standard set: 6 vials (ready to use)	0.25 ml
Progesterone Enzyme Conjugate (20X)	0.7 ml
Progesterone Biotin Conjugate, 1 bottle (ready to use)	7 ml
Assay Diluent, 1 bottle (ready to use)	12 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

MATERIALS NOT PROVIDED

Distilled or deionized water

Precision pipettes

Disposable pipette tips

ELISA reader capable of reading absorbance at 450nm

Absorbance paper or paper towel

Graph paper

STORAGE AND STABILITY

1. 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.
WARNINGS AND PRECAUTIONS	For Invitro use. For laboratory use. Potential biohazardous materials: The calibrator 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. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled. 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.
SPECIMEN COLLECTION HANDLING	Collect blood specimens and separate the serum immediately. Specimens may be stored refrigerated at (2-8 C) for 5 days. If storage time exceeds 5 days, 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. Do not use grossly lipemic specimens.

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REAGENTS PREPARATION	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). 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. Place the desired number of coated strips into the holder Pipet 20 l of Progesterone standards, control and patient's serum samples. Add 100l of working Progesterone Enzyme Conjugate to all wells. Add 50l of Progesterone Biotin Conjugate to all wells. Incubate for 60 minutes at room temperature (20-25C). Remove liquid from all wells. Wash wells three times with 300 ml of 1X wash buffer. Blot on absorbent paper towels. Add 100 l of TMB substrate to all wells. Incubate for 15 minutes at room temperature. Add 50 l of stop solution to all wells. Shake the plate gently to mix the solution. 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: 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. 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.

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)	2.64
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

LIMITATIONS OF THE TEST

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

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