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Product Information

Mouse/Rat Progesterone ELISA

Catalog Number **SE120087**
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 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 10–20 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 is used for the quantitative measurement of progesterone in mouse/rat serum or plasma. It 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.

Components

Materials Provided	96 Tests
Microwell coated with Progesterone MAb	12 x 8 x 1
Progesterone Standard set: 7 vials (ready to use)	0.5 mL
Enzyme Conjugate (20x)	0.7 mL
Assay Diluent	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

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.

20× Enzyme Conjugate
Prepare 1× working solution by diluting 20-fold with assay diluent as needed (*e.g.*, 0.1 mL of the 20× Enzyme Conjugate in 1.9 mL of Assay Diluent is sufficient for 20 wells). The diluted conjugate has to be used the same day.

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

Storage/Stability
Store the kit at 2–8 °C.

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

Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential**.**

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 20 µL of Progesterone standards, control, and serum samples
3. Add 100 µL of Progesterone Enzyme Conjugate to all wells.
4. Incubate for 60 minutes at room temperature (18–26 °C) with shaking.
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

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

Standard	**Optical Units (450 nm)**
Standard 1 (0 ng/mL)	2.85
Standard 2 (0.625 ng/mL)	2.43
Standard 3 (1.25 ng/mL)	1.97
Standard 4 (2.5 ng/mL)	1.40
Standard 5 (5 ng/mL)	0.95
Standard 6 (10 ng/mL)	0.52
Standard 7 (20 ng/mL)	0.29

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