PROGESTERONE

RODENT PROGESTERONE ELISA KIT

INTENDED USE

The Microwell Progesterone ELISA is an enzyme immunoassay system for quantitative determination of progesterone levels in serum/plasma. The test is intended for professional use as a research tool in the monitoring of conditions related to serum progesterone in rodent and related species.

INTRODUCTION

Progesterone is a steroid hormone (C21 steroid, pregn-4-ene-3, 20 dione) and is synthesized from both tissue and circulating cholesterol. The principal production sites are the adrenals and ovaries and placenta during pregnancy. The majority of this steroid is metabolized in the liver to pregnanediol and conjugated as a glucuronide prior to excretion by kidneys.

The primary role is played in reproductive organs. In males the progesterone plays an intermediary role in the synthesis of corticosteroids and androgens. In females, progesterone remains relatively constant through follicular phase of ovulation. Then the levels increase following ovulation and remains elevated for several hours and decreasing to the base line 24 hours before the onset of cycle. In pregnancy, placental progesterone raise 10-30 times those of the luteal peak levels.

In female rodents, the measurement of progesterone is an useful marker in evaluating the status of ovarian functions and also pregnancy.

TEST PRINCIPLES

The progesterone quantitative Test is based on a solid-phase enzyme immunoassay based on competitive binding method. A sample (serum/ plasma/urine) containing an unknown amount of progesterone will compete with enzyme-conjugated progesterone for high affinity binding sites on a limited number of antibodies coated on to the plate. After washing away the free antigen, the amount of labeled antigen in the sample is reversibly proportional to the concentration of the unlabeled antigen. The actual concentrations in unknown samples are obtained by means of a standard curve based on known concentrations of unlabeled antigen analyzed in parallel with the unknowns. After washing, substrate solution is added and the enzyme allowed to react for a fixed time before the reaction is terminated. Absorbencies are measured at 450 nm using ELISA plate reader. A standard curve is produced using values from standards from which absorbency values for blank tubes have been subtracted. Results for unknown may be read directly from this standard curve using either manual calculation or by a suitable computer program. This kit is suitable for the direct measurement of progesterone in serum/plasma samples. It may also be used following an extraction procedure, for assaying urinary progesterone.

MATERIALS PROVIDED	
1. Wells coated with progesterone antibody (96 wells)	
2. Enzyme Conjugate, 12 mL	
3. Sample/standard diluent 25mL	
4. TMB Color Reagent, 12 mL	
5. Stop Solution (2N HCL), 6 mL.	
6. 20 X Wash Buffer, 20mL.	
7. Progesterone Standard Set: 0, 1.0, 2.5, 5.0, 10, 30 ng/mL 0.6 ml /Vial	
QC1 (~2.5 ng/mL and QC2 , 10 ng/mL) 0.6mL/Vial	
8. Instructions	
Materials Required, But Not Provided	
1.	Semiautomatic pipettes: 20ul and 200ul
2.	Disposable pipette tips
3.	Microtiter plate shaker
4.	Microtiter well reader.
5.	Plate washer
6.	Absorbant paper
7.	37 C incubator
8.	Parafilm to cover plate
9.	Distilled water

PRECAUTIONS

1. CAUTION: This kit contains reagents manufactured from blood components and all blood products and samples should be considered potentially infectious and handling should be in accordance with the procedures defined by an appropriate your biohazard safety guideline or regulations.
2. The contents of this kit, and their residues, must not come into contact ruminating animals.
3. Avoid contact with the Stopping Reagent. It may cause skin irritation and burns.
4. Do not use reagents after expiration date.
5. Do not mix or use components from the kits with different lot numbers.
6. Replace caps on reagents immediately. Do not switch caps.
7. Reagents contain sodium azide (NaN3) as a preservative.
   On disposal, flush with a large volume of water to prevent azide build-up.
8. Do not pipette reagents by mouth.
9. Do not use reagents from other kits or mix with other manufactured test kits.

STORAGE & STABILITY CONDITIONS

1. Store the kit at 2-8°C upon receipt and when it is not in use. **Do not Freeze.**
2. Keep microtiter wells in a sealed bag with desiccants to minimize exposure to damp air.
3. Allow all the reagents to reach to room temperature before setting up the assay.
4. Remove only desired number of wells and seal the bag and store at 4-8°C as before.
5. Do not at any time mix or use components with other manufacturer kits. Do not use the kit components after expiration date.

INSTRUMENTATION

A microtiter well reader with bandwidth of 10 nm or less and an optical density range of 0 to 3 OD or greater at 405 nm wavelength is acceptable for use in absorbency measurement.

SPECIMEN COLLECTION AND PREPARATION

1. This kit is suitable for use with serum or heparin plasma samples. The use of hemolytic or lipemic samples will affect results and also samples with bilirubin may interfere with the assay.
2. No special preparation of the samples is required. A venous blood sample (enough to produce about 0.5 ml serum) is collected aseptically.
3. If the sample is not tested immediately refrigerate at 2-8°C. If the storage period greater than 3 days are anticipated, the specimen should be frozen and repeated thawing and freezing should be avoided.
4. If the sample is turbid or contain precipitate may give false results. Such samples should be centrifuged before use. Serum samples with gross lipemia, hemolysis and turbidity should not be used.

REAGENT PREPARATION

1. Prepare Wash buffer by diluting 1 part with 19 parts of distilled water, excess amount may be stored at 2-8 C for couple of weeks.
2. Standard solutions if not used for more than a week, should be kept frozen at -20C.
3. **Sample should be diluted using sample diluent, (eg: 0.05ml sample and 0.2mL sample diluent) and diluted samples should be kept frozen at –20C for further use. This dilution may be an example one should work out the range to fit the sample in the standard range. This will also help you to avoid adding a very small volume (10 ul) which will lead to errors.**

ASSAY PROCEDURE

1. All reagents should be allowed to reach room temperature (18-25C) before use.
2. Pipette 50 ul of standards (ready to use) and diluted samples into appropriate wells within 5 minutes.
3. Add 100ul of progesterone Enzyme Conjugate Solution to each well (except those set for blanks). Mix well for 30 sec. and incubate for 60 minutes at 37°C. You may use parafilm to cover the wells or use appropriate zip-lock bag to store the plate during the incubation.
4. Discard the contents of the wells and wash the plate 5 times with Wash Solution (250-300ul) per well. Invert plate, tap firmly against absorbent paper to remove any residual moisture.
5. Add 100 ul (TMB) Substrate solution to all wells. Remember to follow the pipetting order.
6. Incubate the plate at room temperature (18-28°C) for 10 minutes without shaking.
7. Stop reaction by adding 50ul of Stopping Solution to wells in the same sequence that the Substrate Solution was added and gently mixed.
8. Read the absorbance at 450 nm with a microwell reader.

NOTE: The substrate incubation should be carried out within the temperature range 20-25C. For temperature outside this range, the duration of the incubation should be adjusted.

CALCULATIONS

1. Calculate the mean absorbance values (A) for each set of reference standards, controls, samples and blanks.
2. Subtract the value for blanks from those for standards, control and unknown samples.
3. Calculate the B/B)% values by dividing each value by the value for the zero-standard.
4. For the standards, plot a graph on semi-log graph paper with B/BO% values on the ordinate and the progesterone concentrations (ng/mL) on the abscissa.
5. Using the graph read off the progesterone concentrations for the unknown samples.
6. The values above the readable and below the readable range should be repeated using appropriate dilution.

QUALITY CONTROL

The sensitivity of the assay is 0.1 ng/mL and each laboratory should establish its own base levels based on the species and physiological situation.

Good Laboratory practice requires that quality control specimens be run with each standard curve to establish assay performance characteristics such as recovery, linearity, precision and specificity.

LIMITATIONS OF THE TEST

1. The present Endocrine's ELISA system designed here is for estimation of progesterone levels in serum/plasma samples by a professionally trained laboratory personal only.
2. The wells should be adequately washed to obtain reproducible results. The washing step is extremely important and should be followed according to the instructions.

BIBLIOGRAPHY

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Rodent Progesterone QC Data

Quality Control Data:
It is highly recommended that each laboratory must establish their own internal controls and normal reference values for desired pharmacological and physiological parameters.

A typical standard curve (illustration only) for rodent Progesterone is given below:

Standard ng/mL	OD at 450nm
10	0.05
5.0	0.24
2.5	0.64
1.0	0.96
0.5	1.5
0	2.6

ELISA Performance Characters

Precision: Inter and Intra assay variation (CV) was determined from three different pooled serum samples in three different experiments.

Inter-assay variation	Set1: CV= 5.8% (N=10)	Set2: CV= 5.6 % (N=10)	Set3: CV= 4.9 % (N=10)
Intra-assay variation	Set1: CV= 4.6% (N=10)	Set2: CV= 5.2 % (N=10)	Set3: CV= 6.2 % (N=10)

Sensitivity: The lowest level detectable in this assay is 0.1ng/mL of serum or plasma

Specificity: The rodent Progesterone ELISA system utilizes Highly specific Progesterone antibody Coated on to the plate. The cross-reactivity to other related hormones is not detectable under the sensitivity of the assay system.

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