PROGESTERONE

BOVINE PROGESTERONE ELISA KIT

INTENDED USE

The Microwell Progesterone Elisa kit is an enzyme immunoassay system for quantitative determination of progesterone levels in serum/plasma of Bovine and related species. The test is intended for professional use as an aid in the diagnosis and monitoring of conditions related to serum/plasma progesterone.

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 4-6 days 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 females, the measurement of progesterone is useful in evaluating the status of ovarian functions. Monitoring of progesterone therapy and early stage pregnancy evaluations comprise the remainder of progesterone assays. During early ovarian maturation progesterone levels increase progressively in girls, in parallel with increases in gonadotropins. The importance of sequential progesterone measurements for monitoring ovulation induction, particularly in "in vitro" fertilization programs has recently been reported.

The monitoring of LH and progesterone will help the breeders.

TEST PRINCIPLES

The progesterone quantitative Test is based on a solid-phase enzyme immunoassay based on competitive binding method. A sample (serum/ plasma) 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 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.

MATERIALS PROVIDED
1. Wells coated with progesterone antibody (96 wells)
2. Enzyme Conjugate, 12 mL
3. Progesterone Standard Set: 0, 1.0, 2.5, 5.0, 10, 30 ng/mL
4. TMB Color Reagent, 12 mL
5. Stop Solution (2N HCL), 6 mL.
6. 20 X Wash Buffer, 20 mL.
7. 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. 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 or swine.
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 2-8°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.
6. All Standards should be kept frozen, if not used immediately.

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 450 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. Dilute highly concentrated specimen samples with dilution buffer and mix well before use in the assay.
3. **Standard solutions, if not used immediately, should be kept frozen at -20C.**

ASSAY PROCEDURE

1. All reagents should be allowed to reach room temperature (18-25C) before use.
2. Pipette 50 ul of standards, samples, and controls into appropriate wells.
3. Add 100 ul of progesterone Enzyme Conjugate Solution to each well. Shake the plate well for 30 sec. and incubate at 37C for 1 hour. 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 25-28C. 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 0.5ng/mL and each clinical laboratory should establish its own base levels based on the bovine 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 & Warranty

The present ELISA is designed for helping the scientist to analyze test samples only. There are no warranties, expressed, implied or otherwise indicated, which extend beyond this description of this product. Endocrine Technologies, Inc. is not liable for property or laboratory damage, personal injury, or test samples loss, or economic loss caused by this product. Warranty is limited to replacement of similar ELISA Kit damaged during shipment or leaking solutions within 30 days, with written explanation and return of the ELISA product. The analyst should establish the standard curve and a small number of samples before proceeding to analyze a large number of samples.

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 professional 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.
3. The results obtained with this assay should only be used as an adjunct to other diagnostic procedures and information available to the veterinarian.
4. Trained and skilled professional only should perform the assay.

REFERENCES

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Bovine 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 Bovine Progesterone is given below:

Standard ng/mL	OD at 450nm
30	**0.24**
10	**0.64**
5.0	**0.96**
2.5	**1.5**
1.0	**2.3**
0	**2.8**

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 Bovine 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.