Progesterone ELISA Kit

Catalog Number KA2281
96 assays
Version: 08

Intended for research use only
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Introduction

Intended Use

The Progesterone ELISA Kit is an enzyme immunoassay system for quantitative determination of progesterone levels in serum/plasma of bovine species. The test is intended for research use related to serum/plasma progesterone.

Background

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. Much 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 raises 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.

Principle of the Assay

The Progesterone ELISA Kit 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 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.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wells coated with Progesterone antibody</td>
<td>96 wells/plate</td>
</tr>
<tr>
<td>HRP Enzyme Conjugate</td>
<td>12 mL</td>
</tr>
<tr>
<td>Lyophilized Standard Set: 0, 1.0, 2.5, 5.0, 10, 30 ng/mL, reconstitute in 1 mL standard/sample dilution.</td>
<td>1 set</td>
</tr>
<tr>
<td>TMB Color Reagent</td>
<td>12 mL</td>
</tr>
<tr>
<td>Stop Solution (2 N HCl)</td>
<td>6 mL</td>
</tr>
<tr>
<td>20 X Wash Buffer</td>
<td>20 mL</td>
</tr>
<tr>
<td>Standard/Sample diluent</td>
<td>20 mL</td>
</tr>
</tbody>
</table>

Storage Instruction

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

Materials Required but Not Supplied

✓ Semiautomatic pipettes: 20 μL and 200 μL
✓ Disposable pipette tips
✓ Microtiter plate shaker
✓ Plate washer
✓ Absorbent paper
✓ 37°C incubator
✓ Parafilm to cover plate
✓ Distilled water
✓ Microtiter well reader:
  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.
Precautions for Use

For Research Use Only. Not for Diagnostic Purposes.

Please read the protocol carefully before beginning this assay.

✓ 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.

✓ The contents of this kit, and their residues, must not come into contact ruminating animals or swine.

✓ Avoid contact with the Stopping Reagent. It may cause skin irritation and burns.

✓ Do not use reagents after expiration date.

✓ Do not mix or use components from the kits with different lot numbers.

✓ Replace caps on reagents immediately. Do not switch caps.

✓ Reagents contain sodium azide (Na\(_3\)) as a preservative. On disposal, flush with a large volume of water to prevent azide build-up.

✓ Limitation and Warranty

The present ELISA is designed for helping the scientist to analyze test samples from same species only. There are no warranties, expressed, implied or otherwise indicated, which extend beyond this description of this product. Abnova is not liable for property or laboratory damage, personal injury, or test samples loss, or economic loss caused by this product. The analyst should establish the standard curve and a small number of samples before proceeding to analyze a large number of samples.
Assay Protocol

Reagent Preparation

✓ Prepare Wash buffer by diluting 1 part with 19 parts of distilled water, excess amount may be stored at 4-8°C for couple of weeks.
✓ Lyophilized standards should be diluted in 1 mL standard/sample diluent and should be kept at -20°C, if not used immediately.

Sample Preparation

✓ 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.
✓ No special preparation of the samples is required. A venous blood sample (enough to produce about 0.5 mL serum) is collected aseptically.
✓ If the sample is not tested immediately refrigerate at 4-8°C. If the storage period greater than 3 days are anticipated, the specimen should be frozen and repeated thawing and freezing should be avoided.
✓ 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.
✓ Dilute highly concentrated specimen samples with dilution buffer and mix well before use in the assay.

Assay Procedure

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. Pipette 50 μL of standards, samples, and controls into appropriate wells.
3. Add 100 μL of Progesterone Enzyme Conjugate Solution to each well. Shake the plate well for 30 sec. and incubate at 37°C 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 (250-300 μL) per well. Invert the plate, tap firmly against absorbent paper to remove any residual moisture.
5. Add 100 μL 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 50 μL 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 25-28°C. For temperature outside this range, the duration of the incubation should be adjusted.
Data Analysis

Calculation of Results

✓ Calculate the mean absorbance values (A) for each set of reference standards, controls, samples and blanks.
✓ Subtract the value for blanks from those for standards, control and unknown samples.
✓ Calculate the B/B0% values by dividing each value by the value for the zero-standard.
✓ For the standards, plot a graph on semi-log graph paper with B/B0% values on the ordinate and the progesterone concentrations (ng/mL) on the abscissa.
✓ Using the graph read off the progesterone concentrations for the unknown samples.
✓ The values above the readable and below the readable range should be repeated using appropriate dilution.

Performance Characteristics

✓ Quality Control
  The sensitivity of the assay is 0.5 ng/mL and each 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.

✓ Quality Control Data
  It is highly recommended that each laboratory must establish their own internal controls and normal reference values.
  A typical standard curve (illustration only) for Bovine Progesterone is given below:

<table>
<thead>
<tr>
<th>Standard ng/mL</th>
<th>OD at 450nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.24</td>
</tr>
<tr>
<td>10</td>
<td>0.64</td>
</tr>
<tr>
<td>5.0</td>
<td>0.96</td>
</tr>
<tr>
<td>2.5</td>
<td>1.5</td>
</tr>
<tr>
<td>1.0</td>
<td>2.3</td>
</tr>
<tr>
<td>0</td>
<td>2.8</td>
</tr>
</tbody>
</table>

✓ 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)
Resources

References
