LH (Pig) ELISA Kit

Catalog Number KA2344
96 assays
Version: 05

Intended for research use only
Table of Contents

Introduction .................................................................................................................. 3
  Intended Use ..................................................................................................................... 3
  Background ..................................................................................................................... 3
  Principle of the Assay ....................................................................................................... 3

General Information ....................................................................................................... 4
  Materials Supplied ........................................................................................................... 4
  Storage Instruction ......................................................................................................... 4
  Materials Required but Not Supplied ............................................................................... 4

Assay Protocol ................................................................................................................. 5
  Reagent Preparation ....................................................................................................... 5
  Sample Preparation ....................................................................................................... 5
  Assay Procedure ............................................................................................................ 5

Data Analysis .................................................................................................................. 6
  Calculation of Results ...................................................................................................... 6
  Performance Characteristics ............................................................................................. 6

Resources ........................................................................................................................ 7
  References ....................................................................................................................... 7
  Plate Layout .................................................................................................................... 8
Introduction

Intended Use

LH (Pig) ELISA Kit is an immunoassay designed for the quantitative determination of luteinizing hormone (LH) in serum/plasma samples of Swine and related species. The test is intended for professional use as an aid in the monitoring of physiological conditions related to circulating LH.

Background

Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH) are intimately involved in the control of the growth and reproductive activities of the gonadal tissues, which synthesize and secrete male and female sex hormones. The levels of circulating FSH and LH are controlled by these sex hormones through a negative feedback relationship. LH is a glycoprotein secreted by the basophilic cells of the anterior pituitary. Gonadotropin-release hormone (GnRH or LHRH), produced in the hypothalamus, controls the release of LH and FSH from the anterior pituitary. Like other glycoproteins FSH, TSH, and hCG, LH consists of two subunits alpha and beta. All these hormones have structurally similar alpha subunit, unique beta subunit which determine the biological and immunological properties. In the male the hormone binds to Leydig cells and enhance the secretion of male hormone Testosterone. The LH binds to the theca cells and stimulates steroidogenesis in the ovary. Increased intraovarian Estradiol production occurs as follicular maturation advances, thereupon stimulating increased FSH receptor activity and FSH follicular binding. FSH, LH, and Estradiol are therefore intimately related in supporting ovarian recruitment and maturation in females.

Principle of the Assay

LH (Pig) ELISA Kit is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a polyclonal anti-LH antibody for solid phase (microtiter wells) immobilization and a mouse monoclonal anti-LH antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in LH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a two hour incubation period, at 37°C, the wells are washed with water to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 2 N HCl, and the absorbency is measured spectrophotometrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present and is directly related to the amount of unlabeled LH in the sample. By reference to a series of LH standards assayed in the same way, the concentration of LH in the unknown sample is quantified. Don’t use for other species as the matrix effect may interfere in the ELISA assay leading to erroneous results.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody-coated microtiter wells</td>
<td>96 wells/plate</td>
</tr>
<tr>
<td>Lyophilized Standards (0, 1, 2.5, 5, 10, 25 ng/mL), reconstitute in 1 mL standard/sample diluent.</td>
<td>1 set</td>
</tr>
<tr>
<td>Lyophilized QC1 (~2.0 ng/mL), reconstitute in 1 mL standard/sample diluent.</td>
<td>1 vial</td>
</tr>
<tr>
<td>Lyophilized QC2 (~8.0 ng/mL), reconstitute in 1 mL standard/sample diluent.</td>
<td>1 vial</td>
</tr>
<tr>
<td>Enzyme Conjugate Reagent</td>
<td>12 mL</td>
</tr>
<tr>
<td>TMB Color Reagent, ready to use</td>
<td>12 mL</td>
</tr>
<tr>
<td>20X Wash buffer</td>
<td>20 mL</td>
</tr>
<tr>
<td>Stop solution (2 N HCl)</td>
<td>6 mL</td>
</tr>
<tr>
<td>Standard/Sample Diluent</td>
<td>20 mL</td>
</tr>
</tbody>
</table>

Storage Instruction

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as prescribed above.

Materials Required but Not Supplied

- Precision pipettes: 50 μL, 100 μL, 200 μL, and 1.0 mL
- Disposable pipette tips
- Distilled water
- Glass tubes or flasks to prepare TMB Solution
- Vortex mixer or equivalent
- Absorbent paper of paper towel
- Graph paper
- Microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at a 450 nm wavelength is acceptable for use in absorbency measurement.
Assay Protocol

Reagent Preparation

✓ All reagents should be brought to room temperature (18-25°C) before use.
✓ To prepare the wash buffer add one part of the reagent buffer to 19 parts of distilled water. Prepare desired amount and excess solution can be stored (refrigerated) and is stable for one week.
✓ Lyophilized standards should be diluted in 1 mL standard/sample diluent. This can be stored at -20°C, if not used for a long term.

Sample Preparation

Serum should be prepared from a whole blood specimen obtained by acceptable techniques. This kit is for use with serum/plasma samples without additives only.

Assay Procedure

One must follow accurately these steps to ensure correct results. Use clean pipettes and sterile, disposable tips:
1. Secure the desired number of coated wells in the holder.
2. Dispense 50 μL of standards, specimens, and controls into appropriate wells.
3. Dispense 100 μL of Enzyme Conjugate into each well. Shake the plate for 30 seconds. It is very important to shake the plate at this step.
4. Incubate at 37°C incubator for 2 hours.
5. Remove the incubation mixture by dumping plate contents into a waste container.
6. Rinse and dump the microtiter wells five (5) times with wash buffer.
7. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
8. Dispense 100 μL of TMB solution into each well. Gently mix for 10 seconds.
9. Incubate at room temperature for 20 minutes, in the dark.
10. Stop reaction by adding 50 μL of 2 N HCl to each well.
11. Gently mix for 30 seconds. It is important to observe a color change from blue to yellow.
12. Read optical density at 450 nm with a microtiter well reader.

Important note: The wash steps are very critical. Insufficient washing will result in poor precision and falsely elevated absorbency readings.
Data Analysis

Calculation of Results

Calculate the mean absorbency value (A450) for each set of reference standards, specimens, controls and samples. Construct a standard curve by plotting the mean absorbency obtained from each reference standard against its concentration in ng/mL on graph paper, with absorbency values on the vertical or Y axis, and concentrations on the horizontal or X axis. Use the mean absorbency values for each specimen to determine the corresponding concentration of LH in ng/mL from the standard curve.

Performance Characteristics

- Sensitivity and expected values
  Each laboratory must establish its own normal ranges based on sample population. The minimal detectable concentration of Swine Luteinizing hormone by this assay is estimated to be 0.5 ng/mL.
Resources

References

Plate Layout

A B C D E F G H

1 2 3 4 5 6 7 8

9 10 11 12