LH (Rodent) ELISA Kit

Catalog Number KA2332

96 assays

Version: 09

Intended for research use only

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Introduction

Intended Use

The LH (Rodent) ELISA Kit is an immunoassay designed for the quantitative determination of luteinizing hormone (LH) in serum/plasma samples of rodents and related species. The test is intended for professional use as a research tool in the monitoring of physiological/abnormal 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 enhances 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

The LH (Rodent) ELISA Kit is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a mouse anti-LH antibody for solid phase (microtiter wells) immobilization and mouse anti-LH antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in LH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 2 hours incubation, the wells are washed with wash buffer 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.

General Information

Materials Supplied

List of component

Component	Amount
Antibody-coated 96-wells plate	96-wells plate
HRP Conjugate	12 mL
TMB Color Reagent	12 mL
20x Wash buffer	20 mL
Stop solution (2 N HCl)	6 mL
Standard/Sample Diluent	20 mL
Lyophilized standards (0, 1, 2.5, 5, 10, 25, 50 ng/mL), reconstitute in 1 mL Standard/Sample Diluent.	1 set

Storage Instruction

Immediately after receiving the kit, unopened test kits should be stored at 4-8ºC. The microtiter plate should always be kept in a sealed bag with desiccants to minimize exposure to damp air at room temperature. Opened test kits will remain stable until the expiration date shown, provided it is stored as prescribed above. Do not leave any reagents at room temperature more than 3 hours.

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 or paper towel
- ✓ Graph paper
- ✓ Microtiter plate reader with a bandwidth of 10 nm or less, and an optical density range of 0-3 OD or greater at a 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.

- Limitation of the test

  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. 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

1. All reagents should be brought to room temperature (18-25ºC) before use.
2. 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.
3. Lyophilized Standards should be diluted in 1.0 mL Standard/Sample diluent and should be kept at -20ºC, if not used immediately.

Sample Preparation

- ✓ Serum should be prepared from a whole blood specimen obtained by acceptable techniques. This kit is for use with serum or plasma samples only.
- ✓ Concentrated sample should be diluted using Standard/Sample Diluent provided (1:2 or 1:4 ect.)

Assay Procedure

One must follow accurately these steps to ensure correct results. Technician must make sure all the standards must be dissolved completely before assaying. Always 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. If you choose to use lower volume you may need to work out longer incubation time. One must establish incubation conditions that suite to your lab conditions before analyzing lots of samples.
3. Dispense 100 µL of Enzyme Conjugate into each well. Shake for 30 seconds. It is very important to shake the plate at this step.
4. Incubate at 37ºC 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.

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 your laboratory animals. The minimal detectable concentration of rat Luteinizing hormone by this assay is estimated to be about 0.5 ng/mL.

Resources

References

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Plate Layout

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