Rodent LH

Rodent LUTEINIZING HORMONE (LH) ELISA TEST

PRODUCT PROFILE AND INSTRUCTIONS

INTENDED USE
The Rodent LH ELISA test 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/pathological conditions related to circulating LH.

INTRODUCTION
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.

TEST PRINCIPLE
The LH ELISA Test 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 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 a 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 2N HCl, and the absorbency is measured spectrophotometrically at 450nm. 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.

MATERIALS PROVIDED
1. Antibody-coated microtiter wells, 96-well plate
2. Enzyme Conjugate Reagent, 12 mL
3. TMB Color Reagent (ready to use) , 12 mL
4. 20X Wash buffer, 20 mL
5. Stop solution (2N HCl), 6mL
6. Sample Diluent, 20 mL
7. Reference Standard/Ready to use/0.5mL/Vial
   (0, 1, 2.5, 5, 10, 25, 50 ng/mL, store frozen –20C)
8. **Instructions**

MATERIALS REQUIRED, BUT NOT PROVIDED
1. Precision pipettes: 50uL, 100uL, 200uL, and 1.0mL
2. Disposable pipette tips
3. Distilled water
4. Glass tubes or flasks to prepare TMB Solution
5. Vortex mixer or equivalent
6. Absorbent paper of paper towel
7. Graph paper
8. Microtiter plate reader

SPECIMEN COLLECTION AND PREPARATION
Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum or plasma samples only.

STORAGE OF TEST KIT AND INSTRUMENTATION
Unopened test kits should be stored at 4-8^{O}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. A microtiter plate reader with a bandwidth of 10nm or less, with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at a 450nm wavelength is acceptable for use in absorbency measurement.

REAGENT PREPARATION
1. All reagents should be brought to room temperature (18-25^{O}C) 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. Ready to use Standards should be kept frozen, if not used immediately.
4. Concentrated sample should be diluted using sample diluent provided (1:2 or 1:4 ect.,)

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 50ul 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 100ul of Enzyme Conjugate into each well. Mix for 30 seconds. It is very important to have complete mixing at this step.
4. Incubate at 37^{O}C 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 100ul 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 ul of 2N 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 450nm 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.

CALCULATION OF RESULTS

Calculate the mean absorbency value (A450) for each set of reference standards, specimens, controls and patient 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.

EXPECTED VALUES AND SENSITIVITY

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 0.5ng/ml.

REFERENCES

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Quality Control Data:
It is highly recommended that each laboratory must establish their own internal controls and normal reference values for desired age, sex and physiological parameters.
A typical standard curve (illustration only) for rat/mouse LH is given below:

Standard ng/mL	**OD at 450nm**
0	**0.05**
1.0	**0.31**
5.0	**0.62**
10	**0.95**
25	**1.87**
50	**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= 4.3% (N=10)	Set2: CV= 5.8 % (N=10)	Set3: CV= 5.4 % (N=10)
Intra-assay variation	Set1: CV= 4.9% (N=10)	Set2: CV= 5.4 % (N=10)	Set3: CV= 5.9 % (N=10)

Sensitivity: The lowest level detectable in this assay is 0.5 ng/mL of serum or plasma

Specificity: The rat LH ELISA system utilizes monoclonal antibody and high affinity polyclonal antibody to LH. The cross reactivity to other pituitary gonadotropins (rat TSH, FSH is not detectable under the sensitivity of the assay system.