SWINE LH

PRODUCT PROFILE AND INSTRUCTIONS

LYOPHILIZED STANDARDS

INTENDED USE

The Swine LH ELISA test 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 diagnosis and 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 determines 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 a 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 37C, 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 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. Don't use for other species as the matrix effect may interfere in the ELISA assay leading to erroneous results.

MATERIALS PROVIDED

1. Antibody-coated microtiter wells, 96-well plate
2. Lyophilized Standards: 0, 1, 2.5, 5, 10, 25ng
reconstitute in 1mL standard/sample diluent.
3. Enzyme Conjugate Reagent, 12mL
4. TMB Color Reagent (ready to use), 12mL
5. 20X Wash buffer, 20 mL
6. Stop solution (2N HCl), 6mL
7. Standard/Sample Diluent, 20mL

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/plasma samples without additives only.

STORAGE OF TEST KIT AND INSTRUMENTATION

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. A microtiter plate reader 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°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. Lyophilized standards should be diluted in 1mL standard/sample diluent. This can be stored at -20*C, if not used for long term.

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 100ul of standards, specimens, and controls into appropriate wells.
3. Dispense 100ul 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 37C 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 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 patient population. The minimal detectable concentration of Swine Luteinizing hormone by this assay is estimated to be 0.5ng/ml.

Limitations & Warranty

The present Swine LH 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. 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 or reagents damaged during shipment or leaking solutions or any other issues within 30 days, with written explanation, with sharing the entire data including raw printouts from the reader 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. Endocrine will not replace or refund, only if deemed necessary on scientific merits within 30 days only from the date shown on the shipment date.

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