PRODUCT PROFILE AND INSTRUCTIONS

INTENDED USE

The Equine LH ELISA test is an immunoassay designed for the quantitative determination of luteinizing hormone (LH) in serum/plasma samples of Equines 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 Monoclonal LH antibody for solid phase (microtiter wells) immobilization and a Monoclonal LH beta 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 of 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. Reference Standard/Ready to use 0.5 mL/Vial
   (0, 0.5, 1.0, 2.5, 10, 25,ng/mL., store frozen –20C)
3. Enzyme Conjugate Reagent, 12 mL
4. TMB Color Reagent (ready to use) , 12 mL
5. 20X Wash buffer, 20 mL
6. Sample diluent, 20 ml
7. Stop solution (2N HCl), 6mL
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 adds 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.

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 100 ul of standards, specimens, and controls into appropriate wells. **You may need to add different volumes of your sample to fall on the right concentration of the curve. One must establish sample volume conditions that suite to your lab conditions before analyzing lots of samples., use sample diluent provided to dilute high LH test samples.**
3. Dispense 100 ul of Enzyme Conjugate into each well. Shake the plate for 30 seconds. It is very important to shake the plate very well 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 test 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.

Limitations & Warranty

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. Endocrine Technologies, Inc. is not liable for property or laboratory damage, personal injury, or test sample loss, or economic loss caused by this product. Warranty is limited to replacement of similar ELISA Kit damaged during shipment or leaking solutions within 30 days, with written explanation 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.

EXPECTED VALUES AND SENSITIVITY

Each laboratory must establish its own normal ranges based on your laboratory animals. The minimal detectable concentration of Equine Luteinizing hormone by this assay is estimated to be about 1 ng/ml.

REFERENCES

1. Marshall J.C. Clinic in Endocrinol. Metab. 1975; 4:454
2. Cohen K.L. Mtabolism 1977; 26:1165
3. Rebar R.W., Erickson G.F. and Yen S.S.C. Fertil. Steril. 1982; 37:35
4. Abraham G.E. Ed. Radioassay Systems in Clinic. Endocrinol. Marecel Dekker, Inc., New York (1981)
5. Uotila M., Ruoslahti E. and Engvall E. J. Immunol. Methods 1981; 42:11
6. Harris, G.W. and Naftolinf. The hypothalamus and control of ovulation. Brit. Med. Bullet. 26: 1-9; 1970
7. Shome, B. and Parlow, A.F. J. Clin. Endocrinol. Metabl. 39:199-205; 1974
8. Stabenfeldt, GH., and Hughes JP., Clinical aspects of reproductive endocrinology in the horse 1986. Comend.Contin.Edu.Practicing.Vet 9, 677-686
9. Fonda et.al 1988 A comparison of LH and Progesterone secretion in young and aged mares. J.Anim. Sci. (suppl)1, 65 p429
10. Kindall et al. 1982 Progesterone, Prostaglandin F2 alpha, PMSG, and Estrone sulfate during early pregnancy in mares 1982. J. Repro.Fertil.(suppl) 32, p353-354
11. Ginther OJ. 1979 Reproductive biology of the mare:basic and applied aspects. Cross plaines. WI equiservices.
12. Autrere MB and Benson H 1976 Progesterone: an overview and recent advances Jour Par Sci 65, (6)783-800
13. Chattoraj SC 1976 Endocrine function in Fundamentals of Clinical Chemistry, NW Tietz eds WB Saunders Chap 13, 699-823

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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 Equine LH is given below:

Standard ng/mL	**OD at 450nm**
0	**0.05**
1.0	**0.31**
2.5	**0.48**
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= 5.9% (N=10)	Set2: CV= 6.4 % (N=10)	Set3: CV= 4.4 % (N=10)
Intra-assay variation	Set1: CV= 8.9% (N=10)	Set2: CV= 5.4 % (N=10)	Set3: CV= 8.4 % (N=10)

Sensitivity: The lowest level detectable in this assay is 1 ng/ml of serum or plasma

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