

**Luteinizing Hormone (LH)
ELISA Kit Protocol**

(Cat. No.:EK-310-31)

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INTENDED USE

For the quantitative determination of luteinizing hormone (LH) concentration in human serum. **FOR RESEARCH ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES**

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

TABLE OF CONTENT

Introduction and Protocol Overview	4
Principle of the Test	5
List of Components	6
Specimen Collection and Preparation	6
Storage	6
Reagent Preparation	6
Assay Procedure	7
Calculation of Results	7
Example of Standard Curve	8
Expected Values and Sensitivity	9
References	9

INTRODUCTION AND PROTOCOL OVERVIEW

Luteinizing hormone (LH) is produced in both men and women from the anterior pituitary gland in response to luteinizing hormone-releasing hormone (LH-RH or Gn-RH), which is released by the hypothalamus. LH, also called interstitial cell-stimulating hormone (ICSH) in men, is a glycoprotein with a molecular weight of approximately 30,000 daltons. It is composed of two noncovalently associated dissimilar amino acid chains, alpha and beta. The alpha chain is similar to that found in human thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), and human chorionic gonadotropin (hCG).

The differences between these hormones lie in the amino acid composition of their beta subunits, which account for their immunological differentiation.

The basal secretion of LH in men is episodic and has the primary function of stimulating the interstitial cells (Leydig cells) to produce testosterone. The variation in LH concentrations in women is subject to the complex ovulatory cycle of healthy menstruating women, and depends on a sequence of hormonal events along the gonado-hypothalamic-pituitary axis. The decrease in progesterone and estradiol levels from the preceding ovulation initiates each menstrual cycle. As a result of the decrease in hormone levels, the hypothalamus increases the secretion of gonadotropin-releasing factors (GnRF), which in turn stimulates the pituitary to increase FSH production and secretion. The rising FSH levels stimulate several follicles during the follicular phase, one of these will mature to contain the egg. As the follicle develops, estradiol is secreted, slowly at first, but by day 12 or 13 of a normal cycle increasing rapidly. LH is released as a result of this rapid estradiol rise because of direct stimulation of the pituitary and increasing GnRF and FSH levels. These events constitute the pro-ovulatory phase.

Ovulation occurs approximately 12 to 18 hours after the LH reaches a maximum level. After the egg is released, the corpus luteum is formed which secretes progesterone and estrogen feedback regulators of LH.

The luteal phase rapidly follows this ovulatory phase, and is characterized by high progesterone levels, a second estradiol increase, and low LH and FSH levels. Low LH and FSH levels are the result of negative feedback effects of estradiol and progesterone on the hypothalamic-pituitary axis. After conception, the developing embryo produces hCG, which causes the corpus luteum to continue producing progesterone and estradiol. The corpus luteum regresses if pregnancy does not occur, and the corresponding drop in progesterone and estradiol levels results in menstruation. The

hypothalamus initiates the menstrual cycle again as a result of these low hormone levels.

Patients suffering from hypogonadism show increased concentrations of serum LH. A decrease in steroid hormone production in females is a result of immature ovaries, primary ovarian failure, polycystic ovary disease, or menopause; in these cases, LH secretion is not regulated. A similar loss of regulatory hormones occurs in males when the testes develop abnormally or anorchia exists. High concentrations of LH may also be found in primary testicular failure and Klinefelter syndrome, although LH levels will not necessarily be elevated if the secretion of androgens continues. Increased concentrations of LH are also present during renal failure, cirrhosis, hyperthyroidism, and severe starvation.

A lack of secretion by the anterior pituitary may cause lower LH levels.

As may be expected, low levels may result in infertility in both males and females. Low levels of LH may also be due to the decreased secretion of GnRH by the hypothalamus, although the same effect may be seen by a failure of the anterior pituitary to respond to GnRH stimulation. Low LH values may therefore indicate some dysfunction of the pituitary or hypothalamus, but the actual source of the problem must be confirmed by other tests.

In the differential diagnosis of hypothalamic, pituitary, or gonadal dysfunction, assays of LH concentration are routinely performed in conjugation with FSH assays since their roles are closely interrelated. Furthermore, the hormone levels are used to determine menopause, pinpoint ovulation, and monitor endocrine therapy.

PRINCIPLE OF THE TEST

The LH Quantitative Test is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes a mouse monoclonal anti-alpha-LH antibody for solid phase (microtiter wells) immobilization and a mouse monoclonal anti- β -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 45-minute incubation at room temperature, the wells are washed with water to remove unbound-labeled antibodies. A solution of TMB Reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of LH is directly proportional to the color intensity of the test sample.

LIST OF COMPONENTS

Materials Provided with the Kit:

- Mouse monoclonal anti-alpha LH antibody coated microtiter plate with 96 wells.
- Enzyme Conjugate Reagent, 13 ml.
- LH reference standards, containing 0, 5, 15, 50, 100 and 200 mIU/ml. (WHO, 1st IRP, 68/40), Lyophilized.
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 11 ml.

Materials required but not provided:

- Precision pipettes and tips, 50 μ l, 100 μ l and 1.0 ml.
- Distilled water.
- Disposable pipette tips.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- A microtiter plate reader at 450 nm wavelength, with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater.
- Graph paper.

SPECIMEN COLLECTION AND PREPARATION

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

CAUTION: Phoenix Pharmaceuticals guarantees that its products conform to the information contained in this publication. The purchaser must determine the suitability of the product for its particular use and establish optimum sample concentrations.

STORAGE

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 described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement. **DO NOT FREEZE**

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. Reconstitute each lyophilized standard with 1.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. Reconstituted standards will be stable for up to 30 days when stored sealed at 2-8°C.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 50 µl of standard, specimens, and controls into appropriate wells.
3. Dispense 100 µl of Enzyme Conjugate Reagent into each well.
4. Gently mix for 30 seconds. It is very important to have a complete mixing in this step.
5. Incubate at room temperature (18-25°C) for 45 minutes.
6. Remove the incubation mixture by flicking plate contents into sink.
7. Rinse and flick the microtiter wells 5 times with **distilled or deionized water.** (Please do not use tap water.)
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100 µl TMB Reagent into each well. Gently mix for 10 seconds.
10. Incubate at room temperature in the dark for 20 minutes.
11. Stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix for 30 seconds. **It is important to make sure that all the blue color changes to yellow color completely.**
13. Read the optical density at 450 nm with a microtiter plate reader **within 15 minutes.**

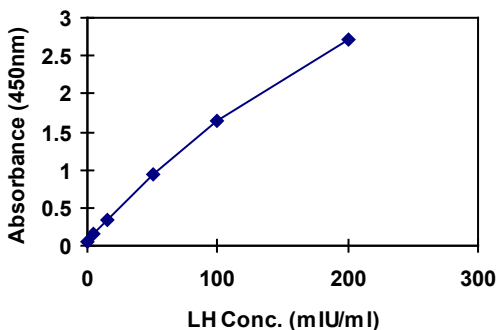
CALCULATION OF RESULTS

1. Calculate the average absorbance values (A₄₅₀) for each set of reference standards, control, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in mIU/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of LH in mIU/ml from the standard curve.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density readings at 450nm shown in the Y axis against LH concentrations shown in the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve in each experiment.

LH (mIU/ml)	Absorbance (450 nm)
0	0.043
5	0.148
15	0.328
50	0.947
100	1.656
200	2.704



EXPECTED VALUES AND SENSITIVITY

Each laboratory must establish its own normal ranges based on patient population. The information provided below is cited from Reference #6:

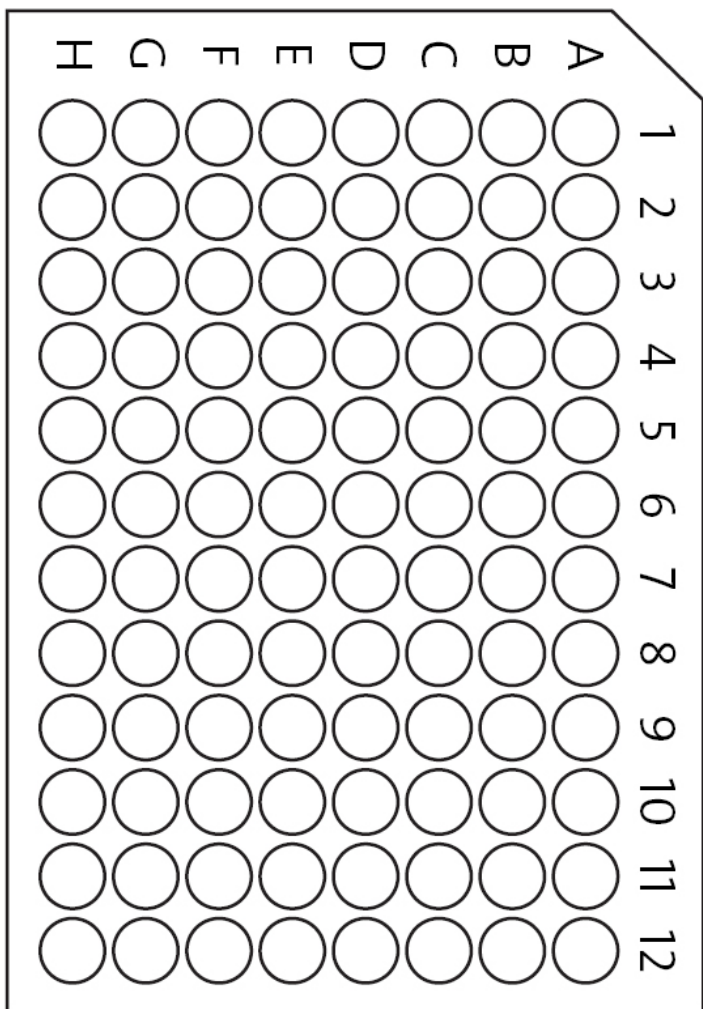
Adult	mIU/ml
Male	1.24-7.8
Female	
Follicular phase:	1.68-15
Ovulatory peak:	21.9-56.6
Luteal phase:	0.61-16.3
Postmenopausal:	14.2-52.3

The minimal detectable concentration of human luteinizing hormone by this assay is estimated to be 1 mIU/ml.

REFERENCES

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5. Uotila, M., Ruoslahti, E. and Engvall, E. *J. Immunol. Methods.* 42:11-15; 1981.
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ASSAY DIAGRAM



NOTES