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# LUTEINIZING HORMONE (LH)

REF

Catalog No.C29-843

IVD

## For In Vitro Diagnostic Use Only

Chemiluminescence Enzyme Immunoassay for the Quantitative Determination of LUTEINIZING HORMONE (LH) in Human Serum

### INTRODUCTION

Chemiluminescence Immunoassay (CLIA) detection using Microplate luminometers provides a sensitive, high throughput, and economical alternative to conventional colorimetric methodologies, such as Enzyme-linked immunosorbent assays (ELISA).

ELISA employs a label enzyme and a colorimetric substrate to produce an amplified signal for antigen, haptens or antibody quantitation. This technique has been well established and considered as the technology of choice for a wide variety of applications in diagnostics, research, food testing, process quality assurance and quality control, and environmental testing. The most commonly used ELISA is based on colorimetric reactions of chromogenic substrates, (such as TMB) and label enzymes.

Recently, a chemiluminescent immunoassay has been shown to be more sensitive than the conventional colorimetric method(s), and does not require long incubations or the addition of stopping reagents, as is the case in some colorimetric assays. Among various enzyme assays that employ light-emitting reactions, one of the most successful assays is the enhanced chemiluminescent immunoassay involving a horseradish peroxidase (HRP) labeled antibody or antigen and a mixture of chemiluminescent substrate, hydrogen peroxide, and enhancers.

Immunospec CLIA Kits are designed to detect glow-based chemiluminescent reactions. The kits provide a broader dynamic assay range, superior low-end sensitivity, and a faster protocol than the conventional colorimetric methods. The series of the kits covers Thyroid panals, such as T3, T4, TSH, Hormone panals, such as hCG, LH, FSH, and other panals. They can be used to replace conventional colorimetric ELISA that have been widely used in many research and diagnostic applications. Furthermore, with the methodological advantages, Chemiluminescent immunoassay will play an important part in the Diagnostic and Research areas that ELISAs can not do.

The CLIA Kits have been validated on the ***MPL2*** microplate luminometer from Berthold Detection System, ***Lus2*** microplate luminometer from Anthos, ***Centro LB960*** microplate luminometer from Berthold Technologies, and ***Platelumino*** from Stratec Biomedical Systems AG. We got acceptable results with all of those luminometers.

### INTRODUCTION OF LH IMMUNOASSAY

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 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 pre-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 ovulatary 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 the 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 dose 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

Immunospec LH Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-LH antibody for solid phase (microtiter wells) immobilization and another mouse 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 the LH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 60 minute incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of chemiluminescent substrate is then added and read relative light units (RLU) in a Luminometer. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of 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 with Test Kit

1. Antibody-coated microtiter plate with 96 wells.

2. Reference standard set, contains 0, 3.0,10.0,30.0,60.0, and 120 mIU/ml.(WHO, 1st IRP, 68/40), lyophilized.
3. Enzyme Conjugate Reagent, 12.0 ml.
4. Wash Buffer Concentrate (20X), 30 ml
5. Chemiluminescence Reagent A, 6.0 ml
6. Chemiluminescence Reagent B, 6.0 ml

## Materials Required but not Provided

1. Distilled water.
2. Precision pipettes: 0.04~0.2ml, 1.0 ml
3. Disposable pipette tips.
4. Glass tube or flasks to mix Reagent A and B.
5. Microtiter well reader.
6. Vortex mixer or equivalent.
7. Absorbent paper.
8. Graph paper.

## Reagent Preparation

1. All reagents should be allowed to reach room temperature (18-25$^{0}$C ) before use.
2. To prepare substrate solution, make an 1:1 mixing of Reagent A with Reagent B right before use. Mix gently to ensure complete mixing. Discard excess after use.
3. Reconstitute each lyophilized standard and controls with distilled water (Reconstitute volume see labels). Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards and controls should be stored sealed at 2-8°C.
4. Prepare the washing solution by diluting 1 part of the 20X Wash buffer concentrate to 19 parts of distilled water.

## Assay Procedure

1. Secure the desired number of coated wells in the holder. Make data sheet with sample identification.
2. Dispense 50µl of standard, specimens, and controls into appropriate wells.
3. Dispense 100µl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to have complete mixing in this setup.
5. Incubate at room temperature (18-25°C) for 60 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the microtiter wells 5 times with washing buffer.
8. Strike the wells sharply onto absorbent paper to remove residual water droplets.
9. Dispense 100 µl Chemiluminescence substrate solution into each well. Gently mix for 5 seconds.
10. Read wells with a chemiluminescence microwell reader 5 minuters later. (between 5 and 20 min. after dispencing the substrates).

**Important Note:**

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. If there are bobbles existing in the wells, the false readings will be created. Please use distilled water to remove the bobbles before adding the substrate.

## Calculation of Results

1. Calculate the average read relative light units (RLU) for each set of reference standards, control, and samples.
2. We recommend to use a proper software to calculate the results. The best curve fitting used in the assays are 4-parameter regrassion or cubic spline regaression. If the software is not available, construct a standard curve by plotting the mean RLU obtained for each reference standard against LH concentration in ng/ml on linear graph paper, with RLU on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of LH in mIU/ml from the standard curve.

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## Example of Standard Curve

Results of a typical standard run are shown below. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. It is required that running assay together with a standard curve each time. The calculation of the sample values must be based on the particular curve, which is runing at the same time.

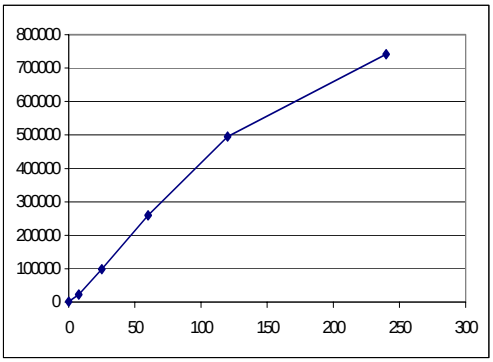
| LH (mIU/ml) | Relative Light Units (RLU) ($10^4$) |
|---|---|
| 0.0 | 0.1 |
| 3.0 | 2.3 |
| 10.0 | 9.9 |
| 30.0 | 26.0 |
| 60.0 | 49.6 |
| 120.0 | 74.2 |

**Expected values and sensitivity**

Each laboratory must establish its own normal ranges based on patient population. The results provided below are based on randomly selected out-patient clinical laboratory samples:

| | LH (mIU/ml) | | | |
|---|---|---|---|---|
| | Age | No. of patients | Mean | Range |
| Male | < 10 | 25 | 1.3 | < 2.5 |
| Male | 15-60 | 56 | 4.8 | 1.0 to 15.0 |
| Female | < 10 | 25 | 1.1 | < 2.0 |
| Female | 20-35 | 60 | 15.0 | 1.0 to 90.0 |
| Female | 46-60 | 40 | 38.0 | 8.0 to 120.0 |

The minimal detectable concentration of human luteinizing hormone by this assay is estimated to be 2 mIU/ml.

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EC REP

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