LH (Human) ELISA Kit

Catalog Number KA0214

96 assays

Version: 02

Intended for research use only
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**Introduction**

**Intended Use**

For the quantitative determination of luteinizing hormone (LH) concentration in human serum.

**Background**

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 non-covalently 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 upon 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, increases 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, the 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 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 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 conjunction 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 Assay**

The LH ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes mouse monoclonal anti-α-LH 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 the LH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 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.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse monoclonal anti-α LH antibody coated microtiter plate</td>
<td>96 wells</td>
</tr>
<tr>
<td>Enzyme Conjugate Reagent</td>
<td>13 ml</td>
</tr>
<tr>
<td>LH reference standard contains 0, 5, 15, 50, 100, and 200 mIU/ml (WHO, 1st IRP, 68/40)</td>
<td>Lyophilized</td>
</tr>
<tr>
<td>TMB Reagent (One-Step)</td>
<td>11 ml</td>
</tr>
<tr>
<td>Stop Solution (1N HCl)</td>
<td>11 ml</td>
</tr>
</tbody>
</table>

Storage Instruction

Unopened test kit should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Opened test will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plat reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

Materials Required but Not Supplied

- Precision pipettes: 50 µl, 100 µl 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.

Precautions for Use

- Limitation of procedures
- 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.
- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
✓ Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

✓ The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.
Assay Protocol

Reagent Preparation

All reagents should be allowed to reach room temperature (18-25°C) before use. 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.

Sample Preparation

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

Assay Procedure

1. Secure the desired number of coated wells in the holder.
2. Dispense 50 µl of standards, 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 complete mixing in this setup.
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 100µl of 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 100µl of Stop Solution to each well.
12. Gently mix for 30 seconds. It is important to make sure that all of the blue color changes completely to yellow color completely.
13. Read optical density at 450nm with a microtiter plate reader within 15 minutes.
Data Analysis

Calculation of Results

- Calculate the average absorbance value ($A_{450}$) for each set of reference standards, controls and samples.
- Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in mIU/ml on linear graph paper, with absorbance on the vertical or Y-axis, and concentration on the horizontal or X-axis.
- 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.

<table>
<thead>
<tr>
<th>LH (mIU/mL)</th>
<th>Absorbance (450nm)</th>
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<tbody>
<tr>
<td>0</td>
<td>0.043</td>
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<tr>
<td>5</td>
<td>0.148</td>
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<tr>
<td>15</td>
<td>0.328</td>
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<tr>
<td>50</td>
<td>0.947</td>
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<tr>
<td>100</td>
<td>1.656</td>
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<td>200</td>
<td>2.704</td>
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Performance Characteristics

- Expected Values
Each laboratory should establish its own normal range based on patient population. The information provided below is cited from Reference #6:

<table>
<thead>
<tr>
<th>Adult</th>
<th>Adult mIU/ml</th>
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<tbody>
<tr>
<td>Male</td>
<td>1.24-7.8</td>
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<td>Female</td>
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<tr>
<td>Follicular phase:</td>
<td>1.68-15</td>
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<td>Ovulatory peak:</td>
<td>21.9-56.6</td>
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<td>Luteal phase:</td>
<td>0.61-16.3</td>
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<td>Postmenopausal:</td>
<td>14.2-52.3</td>
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</table>

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

References

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