Human LH ELISA Kit

Enzyme Immunoassay for the quantitative determination of human Luteinizing Hormone (LH) in serum

Catalog number: ARG80850

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INTRODUCTION

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 difference 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 preceeding 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, corpus luteum is formed which secretes progesterone and estrogen - two 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 hypotalamic-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

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for LH has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any LH present is bound by the immobilized antibody. After washing away any unbound substances, a HRP-conjugated antibody specific for LH is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of LH bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of LH in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 strips x 8-well	4°C
Standards 0-5 (0, 10, 20, 40, 100, 200 mIU/ml)	6 vials (lyophilized)	4°C
HRP-LH antibody Conjugate	11 ml (ready to use)	4°C
TMB substrate	14 ml (ready to use)	4°C (Protect from light)
STOP solution	14 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- Store the unopened reagents at 2 - 8°C until expiration date. Once opened the reagents are stable for 2 month when stored at 2 – 8 °C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.
- All kit reagents and specimens should be brought to room temperature

(21-26°C) and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.

- Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
- Ensure complete reconstitution and dilution of reagents prior to use.
- The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
- Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
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- Pipetting of all standards, samples, and controls should be completed within 6 minutes. (Note this especially for manual pipetting.)

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Remove serum and assay immediately. Sample can be stored at 2-8 °C up to 48 hours. For long-term storage, aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. It is recommended that samples should only be frozen-thawed once.

Note: 1. Thawed samples should be inverted several times prior to testing.
2. Do not use haemolytic, icteric or lipaemic specimens.
3. Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- Standards: Reconstitute the lyophilized contents of the standard with 1 ml distilled water and let the standards stand at least 10 minutes at room temperature. Mix several times before use. The reconstituted standards are stable for 2 months at 2-8°C. For longer storage, aliquot and store at ≤ -20 °C.
- Sample: If the initial assay found samples contain LH higher than the highest standard, the samples can be diluted with Standard 0 and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

Samples should be capped and may be stored for up to 48 hours at 2-8 °C prior to assaying. Samples held for a longer time should be frozen only once at-20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

Example:

a) Dilution 1:10: 10 µL Serum + 90 µL Standard 0 (mix thoroughly).

b) Dilution 1:100: 10 µL 1:10 diluted a) + 90 µL Standard 0 (mix thoroughly).

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 21-26°C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **25 µl** of **Standard, controls and samples** with new disposable tips in duplicate into the appropriate wells.
3. Add **100 µl** of **HRP-LH antibody** into each well. Thoroughly mix for 10 sec. (Mixing completely is important in this step). Incubate for **30 minutes** at **RT**.
4. Aspirate each well and wash, repeating the process 4 times for a **total 5 washes**. Briskly shake out the contents of the wells. Wash by filling each well with distilled water (**400 µl**) using a squirt bottle, manifold dispenser, or autowasher. Rinse the wells 5 times in total. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining distilled water by aspirating, decanting or blotting against clean

paper towels. Strike the wells sharply on absorbent paper to remove residual droplets. The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!

5. Add 100 µl of TMB substrate to each well. Incubate for 10 minutes at room temperature in dark.
6. Add 50 µl of Stop Solution to each well.
7. Read the OD with a microplate reader at 450 nm immediately. It is recommended that the wells should be read within 10 min after adding Stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value 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 from the standard curve.

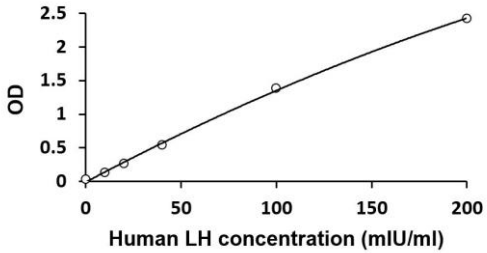
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as

described above.

5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 200 mIU/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The analytical sensitivity was calculated from the mean plus two standard deviations of twenty (20) replicate analyses of Standard 0 and was found to be 1.27 mIU/ml.

Assay Range

1.27-200 mIU/ml

Specificity

The following substances were tested for cross reactivity of the assay:

Hormone Tested	Concentration	Produced Color Intensity Equivalent to LH in Serum (mIU/mL)
hCG (WHO 1st IRP 75/537)	200 mIU/mL	5.2
TSH (WHO 2nd IRP 80/558)	62 µIU/mL	3
FSH (WHO 1st IRP 68/40)	200 mIU/mL	2.5

Note:

Pregnancy results in elevated levels of hCG, the samples used for the LH enzyme immunoassay test with this human LH ELISA kit is not recommended collected during pregnancy or immediately post-partum.

Intra-assay and inter-assay precision

The CV value of intra-assay precision was 5.58% and the CV value of inter-assay precision was 6.23%.

Interferences

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results.

Recovery

89.7-107.4%