LUTEINIZING HORMONE (LH) ENZYME IMMUNOASSAY TEST KIT
Catalog Number: 6107320

CE

Enzyme Immunoassay for the Quantitative Determination of Luteinizing Hormone (LH) Concentration in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2 to 8°C.

INTENDED USE

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

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 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 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 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 tests 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 EIMA TEST is based on simoultaneous binding of human LH to two monoclonal antibodies, one immobilized on microwell plates, the other coniugates with horseradish peroxidase (HPR).
After incubation the bound/free separation is performed by a simple solid-phase washing, then the substrate solution (TMB) is added. After an appropriate time has elapsed for maximum color development, the enzyme reaction is stopped and the absorbance are determinated.

The LH concentration in the sample is calculated based on a series of standard.
The color intensity is proportional to the LH concentration in the sample.
The test is performed as an indirect solid phase sandwich-type immunoassay. Microwells are coated with monoclonal

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anti-LH followed by blocking the unreacted sites to reduce non-specific binding.

Step 1 LH Antigens present in calibrators and patient samples bind to the coated antibody.

Step 2 The Antigen-Antibody complex is reacted with enzyme (HRP) labeled monoclonal anti - LH conjugate resulting in the LH antigen being sandwiched between the solid phase antibody and the enzyme conjugate.

Step 3 The enzyme converts added substrate (TMB) to form a colored solution.

Step 4 The intensity of color change, which is proportional to the concentration of Antigenes present in the samples is read by a microplate-reader at 450 nm. Results are expressed in mIU/mL.

REAGENTS

Materials provided with the kit:

- Microwell plate. 12x8 well strips. Individually separable wells. Coated with anti-monoclonal LH,, packaged in an aluminum bag with a drying agent.
- Calibrators. 5 Vials x 0.4 mL With a concentrations of 0, 5; 20; 75; and 200 mIU/mL.
- Enzyme Conjugate. anti-monoclonal LH -horseradish peroxidase (HRP) conjugate. Ready-to-use. 12 mL.
- Substrate solution. H_2O_2-TMB 0.25 g/L avoid any skin contact). 12 mL
- Stop Solution. Sulphuric acid 0.15 mol/L (corrosive: avoid any skin contact). 12 mL

Materials required but not provided:

- Multichannel pipettes and micropipettes (Precision ≥1.5%) and disposable tips.
- Microplate reader with a 450 nm filter. Reference filter of 620 or 655 nm is advisable.
- Manual or automated wash system.
- Absorbent paper of blotting the microplate wells.
- Distilled or deionised water.
- Timer.

EXPECTED VALUES

Each laboratory must establish its own normal ranges based on patient population.

The serum or plasma LH values are comprised in the following intervals:

WOMAN:	follicular phase	1.5	–	8.0	mIU/mL
	luteinic phase	0.2	–	6.5	mIU/mL
	ovulation phase	5.0	–	24.0	mIU/mL
	menopause	16.0	–	90.0	mIU/mL

STORAGE OF TEST KITS

The components will remain stable through the expiration date shown on the label if stored between 2-8°C in dark. Do not frezee. Do not use reagents beyond the kit expiration date.

PREPARATION

- Coated microwell strips are for one time use only.
- Calibrators, Enzyme Conjugate, Substrate Solution and Stop Solution are ready to use and need not to be diluted.

PRECAUTIONS

- Instructions should be followed exactly as they appear in this kit insert to ensure valid results.
- Avoid contact with the TMB (3,3`,5,5`-Tetramethyl-benzidine). If TMB comes into contact with skin wash thoroughly with water and soap.
- The stop solution contains sulphuric acid. If it comes into contact with skin, wash thoroughly with water and seek medical attention.
- Avoid contact between the buffered peroxide solution and easily oxidized materials; extreme temperatures may initiate spontaneous combustion.
- Do not use beyond expiration date on the label.
- Do not use if reagent is not clear or if a precipitate is present.
- Do not interchange kit components with those from other sources other than the same catalog number from LINEAR.
- Follow good laboratory practices to minimize microbial and cross contamination of reagents when handling.
- All human derived components used have been tested for HBsAg, HCV, HIV-1 and 2 and HTLV-I and found negative by FDA required tests. However, human blood derivatives and patient specimens should be considered potentially infectious. Follow good laboratory practices in storing, dispensing and disposing of these materials.

SPECIMEN COLLECTION AND PREPARATION

Use fresh serum or plasma. Samples can be stored at 2-8°C for 2 days. For longer periods, samples should be frozen (-20°C). Avoid repeated freezing and thawing.

Grossly hemolyzed, lipemic or microbially contaminated specimens may interfere with the performance of the test and should not be used. Neither Bilirubin nor Hemolysis have significant effect on the procedure.

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ASSAY PROCEDURE

Procedural Notes

- Before starting with the assay read carefully the product insert.
- Let specimens and test reagents equilibrate at room temperature before starting with the test procedure. Return all unused specimens and reagents to refrigerator immediately after use.
- Remove required microwell strips from the pouch and carefully reseal the pouch to prevent condensation in the unused wells. Return pouch immediately to refrigerator.
- Good washing technique is critical. For manual washing, fill each microwell with 300 µl distilled water. Discard the fluid by inverting and tapping out the contents of each well or by aspirating the liquid from each well. To blot at the end of the last wash, invert strips and tap the wells vigorously on absorbent paper towels. For automatic washers, program the washer as per manufacturer's instructions.
- Use a multichannel pipette capable of delivering 8 wells simultaneously. This speeds the process and provides for a more uniform incubation time.
- For all steps, careful control of timing is important. The start of all incubation periods begins with the completion of reagent addition.

Preparation of Samples

Usually no dilution necessary; for samples with concentration above 200 mIU/mL, dilute the sample 1:1 with Standard A.

Test procedure

1. Label protocol sheet to indicate sample placement in the wells according to the following figure. 5 calibrators (standards) (SA-SE) and 1 Blank should be included. The user has the option to run Patient Samples (P) in duplicate.

	1	2	3	4	5	6	7	8	9	10	11	12	Cal.	Conc. mIU/mL
a	B	SD	P3										SA	0
b	SA	SE	P4										SB	5
c	SA	SE	P4										SC	20
d	SB	P1	P..										SD	75
e	SB	P1	P..										SE	200
f	SC	P2												
g	SC	P2												
h	SD	P3												

2. Remove the required microwells from pouch and return unused strips in the sealed pouch to refrigerator. Securely place the microwells into the extra provided holder .
3. Pipette 25 µL of Calibrators and 25 µL Patient Samples into the wells.
   Incubate 10 minutes at room temperature
4. Add 100 µL Enzyme Conjugate to the wells except for Blank well and incubate 60 minutes at room temperature.
5. Add app. 300µL of distilled water, decant (tap or blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes.
6. Pipette 100 µL of Substrate Solution into each microwell in the same order and timing as for the Enzyme Conjugate, Blank well included.
7. Incubate 10 minutes at room temperature in the dark.
8. Add 100 µL of Stop Solution into each microwell using the same order and timing as for the addition of the Substrate Solution.
9. Read absorbance of each microwell at 450 nm against blank using a microplate reader. The developed color is stable for at least 30 minutes. Read optical densities during this time.

TEST EVALUATION

Mean absorbance and relative percentage

1. Calculate the mean of the absorbances (Em) corresponding to the single points to the standard curve and of each sample.
2. Subtract the mean absorbance value of the zero standard from the mean absorbance values of standards and samples.
3. Draw the standard curve on log-log or lin-lin graph paper by plotting absorbance values of standard against appropriate LH concentration.
4. Read off the LH concentrations of the control and samples.

LIMITATIONS OF THE PROCEDURE

The assay should not be performed on grossly hemolyzed, microbially contaminated or lipemic samples. This method should be used for testing human serum samples only.

PERFORMANCE CHARACTERISTICS

Sensitivity

The minimal detectable concentration of Human LH by this assay is estimated to be 1.0 mIU/mL.

Specificity

The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

hLH	100.0 %
hFSH	3.0 %
HCG	4.0 %
hTSH	0.02 %

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Precision

a. Intra Assay variation
Within-run precision was determined by replicate determination of three different control in one assay. The within assay variability is shown below:

Sample	1	2	3
Number of replicates	16	16	16
Mean LH (mIU/mL)	3.6	21.2	48.2
Std. Deviation	0.21	1.15	3.02
Coef. of Variation (%)	5.8	5.4	6.25

b. Inter Assay variation
Between-run precision was determined by replicate determination of three different controls in one assay. The between assay variability is shown below:

Sample	1	2	3
Number of replicates	16	16	16
Mean LH (mIU/mL)	3.4	20.6	51.3
Std. Variation	0.26	1.51	4.33
Coef. of Variation (%)	7.6	7.2	8.44

Recovery
Various patient samples of known LH levels were combined and assayed in duplicate. The average recovery 99.5% with reference to the original concentrations.

Expected conc.	Observed conc.	Recovery
8.5	8.8	103.5
14.6	13.7	93.8
45.2	43.8	96.9
68.8	73.4	106.7
125.1	120.9	96.6

Linearity
Two patient samples were serially diluted with zero standard in a linearity study. The average recovery was 102.1 %.

Patient	Exp. Conc	Obs. Conc	Recovery
1		86.4	
Dil. 1 / 2	43.2	44.1	102.8
Dil. 1 / 4	21.6	22.1	102.3
Dil. 1 / 8	10.8	10.4	96.2
2		92.8	
Dil. 1 / 2	46.4	45.5	98.0
Dil. 1 / 4	23.2	24.0	103.4
Dil. 1 / 8	11.6	10.8	93.1

Limitations of the procedure

In this assay, no hook effect is observed up to 4000 mIU/mL of LH.

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