LH ELISA Kit

48 Tests Kit
Enzyme Immunoassay for the Quantitative Determination of Luteinizing Hormone (LH) Concentration in Human Serum/Plasma
(For In Vitro Diagnostic Use Only)
Catalogue No. PT-LH-48

PISHTAZ TEB DIAGNOSTICS

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.
A lack of secretion by the anterior pituitary may cause lower LH levels. As may be expected, low levels may results 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 valued 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 conjunctions with FSH assays since their roles are closely interrelated. Furthermore, the hormone levels are used to determine menopause, pinpoint ovulation, and monitor endocrine therapy.

Test Principle

The Pishtaz Teb 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 with the solid phase antibodies, after incubation and washing, the enzyme conjugate will be added, resulting in the sandwich formation of LH between solid phase and conjugated antibodies. After second wash step a solution of TMB is added and incubated for 15 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 1M HCl, 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.

Materials provided with the kit

1. Antibody coated wells (1 plate, 48 wells): Microtiter wells coated with monoclonal anti hLH.
2. Enzyme conjugate (1vial, 3 ml): Monoclonal anti hLH labeled with HRP in buffer containing protein as stabilizer and Thiomersal as preservative, ready to use.
3. Standard set (0.5 ml / vial): Contains 0.0 (1 ml), 2.5, 5, 25, 50 and 100 IU/L of LH calibrated against WHO 2nd International Standard code 80/552 in buffer containing protein as stabilizer and thiomersal as preservative, ready to use.
4. Control serum (1 vial, 0.5 ml): Contains certain amount of human LH in buffer containing protein as stabilizer and Thiomersal as preservative, ready to use.
5. Chromogen substrate reagent (1 vial, 6ml):

Contains Tetra Methyl Benzidine (TMB) and hydrogen peroxide, ready to use.
6. Wash solution(1 vial, 25 ml concentrated 20x): Contains phosphate buffer solution with 0.05 % Tween 20.
7. Stop solution (1 vial, 6 ml): Contains Hydrochloric acid (1M)
8. Cardboard sealer.

Materials required but not provided

1. Precision pipettes: 50µl, 100µl
2. Disposable pipette tips.
3. Distilled water.
4. Absorbent paper or paper towel.
5. Graph paper.
6. Microtiter plate reader.

General Information

1. Do not mix kit reagents from different batch/lot numbers. All kit components must be used only in their original kit.
2. All reagents obtained from human sources are negative for HBs Ag, HCV and HIV antibodies. To prevent risk of contamination, use personal protective equipments like gloves, lab coats, etc. and avoid direct contact with reagents.

Storage Conditions

1. Kit should be stored at 2-8 °C upon receipt and when it is not in use.
2. Keep Un-used wells in their sealed bag with desiccants.
3. Do not use expired date reagents.

Specimen Collection and Preparation

The kit is for use with serum or plasma. Serum or plasma should be prepared from a whole blood specimen obtained by approved aseptic technique. If testing cannot be done within an hour after sample collection, refrigerate (maximum 48 hours) the specimen immediately and let it return to room temperature before testing. If prolong storage is required, samples should be stored at -20 °C (maximum 30 days). Avoid freeze-thaw of specimen during storage.

Reagents Preparation

1. All reagents should be allowed to reach room temperature (22-28ºC) before use.
2. Working wash solution: dilute concentrated wash solution 1:20 with distilled water before use.

Test Procedure

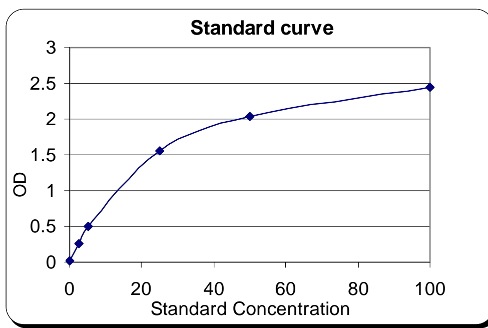
1. Secure the desired number of coated wells in the holder and keep the remaining with desiccants in tightly closed special bag.
2. Dispense 50 µl of each standard, serum control and specimen in appropriate wells.
3. Cover the microtiter wells with cardboard sealer. Leave wells for 30 minutes at room temperature (22-28ºC).
4. Remove the sealer and take out wells contents by flicking the microplate into a waste container. Rinse and flick the microtiter wells 5 times (each time with 300 µl of working wash solution). Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
5. Add 50 µl of Anti-LH-HRP conjugate into the wells.
6. Seal the plate with cardboard sealer again. Incubate wells for 30 minutes at room temperature (22-28ºC).
7. Repeat step 4.
8. Dispense 100 µl of chromogen/ substrate soution into the microplate wells.
9. Leave the microplate wells at room temperature and dark for 15 minutes, to develop color.
10. Stop the reaction by adding 100 µl of stop solution to the microplate wells.
11. Measure absorbance at 450 nm by ELISA reader (Use 630 nm filter as reference filter if it's available).

Result Calculation

1. Calculate mean absorbance value of standards and samples at 450 nm (Use 630 nm filter as reference filter if it's available)
2. Construct a point to point standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in IU/L on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Use the mean absorbance value for each sample, determine the corresponding concentration of LH in IU/L from the standard curve.

Example of Standard curve

Standards (IU/L)	OD
0	0.02
2.5	0.26
5	0.50
25	1.55
50	2.03
100	2.45



Note: All absorbances shown in above curve are 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.

Expected Values

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

	Mean (IU/L)	Reference interval (IU/L)
Adult females		
Follicular phase	6	1-11
Mid-cycle	34	10-80
Luteal phase	7	1-14
Post-Menopause	24	8.1-44
Males under 60 y/o	4.8	1.2-8.6

Performance Characteristics

1. Minimum Detection Limit

Based on mean absorbance of zero standard +3SD the minimum detectable concentration of LH by this assay is estimated to be 1 IU/L.

2. Test Precision

To assess precision of this assay kit, three serum samples with different concentrations of LH were repeatedly tested.
Results are shown in table 1 and 2:

Table 1: Intra-assay

No.	No. of tests performed	Means IU/L	SD IU/L	CV%
1	24	6.1	0.3	5.4
2	24	22	1.0	4.5
3	24	55	2.4	4.3

Table 2: Inter-assay

No.	No. of tests performed	Means IU/L	SD IU/L	CV%
1	10	5.7	0.4	7.3
2	10	20.8	0.9	4.3
3	10	54.7	3.8	6.9

Each test has been run in duplicate

3. Test Recovery

The certain amount of hLH was added into 4 different serum samples with known concentration of hLH and their recovery were determined. The results are shown below:

Table 3: Test recovery

No.	hLH level IU/L	hLH added IU/L	Exp. IU/L	Obs. IU/L	Rec. (%)
1	12	5	8.5	8	94
1	12	25	18.7	19.5	104
1	12	50	31	30	97
2	29	5	17	18.5	109
2	29	25	27	26	96
2	29	50	39.5	41	103
3	44	5	24.5	25.5	104
3	44	25	34.5	33	96
3	44	50	47	49	104
4	62	5	33.5	34	101
4	62	25	43.5	41.5	95
4	62	50	56	58	103

Exp.: Expected, Obs.: Observed,
Rec.: Recovery

4. Test Linearity

To verify test linearity, 6 different serum samples with known hLH concentration were sequentially diluted with 0 standard reagent. Then the serums were tested with LH ELISA test and the results are shown below (considering dilution factor):

Table 4: Test linearity

		Recovery (%)			
No.	hLH undiluted specimen	1:2	1:4	1:8	1:16
1	8.5	98	104	99	-
2	24	99	102	95	97
3	32	100	96	104	94
4	39	98	94	103	97
5	48	101	107	95	96
6	87	96	98	100	98

5. Test Specificity

To determine cross reaction of LH ELISA kit with closely related kits, the following hormones were tested: hFSH, hTSH and hCG. Results are shown in the following table:

Table 5: Specificity test result for cross reaction

Analyte name	Concentration (IU/L)	Apparent hLH level
hFSH (IU/L)	500 250 100 50	< 1.0
hTSH (mIU/L)	500 250 100 50	< 1.0
hCG (IU/L)	100000 10000 1000 100	< 1.0

6. Hook effect

To rule out possible hook effect occurrence, the hLH assay was done on serums with high concentration of hLH (up to 2500 IU/L) and no “hook effect” was seen.

Reference

Wide, L., Loraine, Ed. J. A., Bell, E. T. (1976) Human pituitary gonadotropins: hormone assays and their clinical application, Churchill Livingstone, Edinburgh, London and New York, 4 ed. pp 78-140.

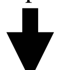
LH Test Procedure

Step 1

Reagent	Standard	**Serum Control**	**Sample**
Standard	50 µl	None	None
Control Serum	None	50 µl	None
Sample	None	None	50 µl

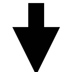
Cover the microplate wells with cardboard sealer tightly and incubate them for 30 minutes at room temperature.

Step 2



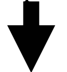
Remove plate cover and discard contents of the wells. Wash the microplate wells for 5 times according to test manual.

Step 3



Anti LH-HRP conjugate	50 µl	50 µl	50 µl

Cover the microplate wells again with cardboard sealer and incubate them for 30 minutes at room temperature.



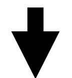
Step 4

Remove plate cover and discard contents of the wells. Wash the microplate wells for 5 times according to test manual.

Chromogen -substrate solution	100 µl	100 µl	100 µl

Incubate wells for 15 minutes at room temperature and dark.

Step 5



Stop Solution	100 µl	100 µl	100 µl

Read absorbance at 450 nm (Use 630 nm filter as reference filter if it's available.