POINTE SCIENTIFIC
A MEDTEST COMPANY

Luteinizing Hormone (LH) Enzyme Immunoassay Test Kit

ENZYME IMMUNOASSAY FOR THE QUANTITIVE DETERMINATION OF LUTENIZING HORMONE (LH) IN HUMAN SERUM

FOR IN VITRO DIAGNOSTIC USE

Store at 2 to 8°C.

INTENDED USE

The LH ELISA is intended for the quantitative determination of luteinizing hormone (LH) in human serum. This assay is useful in the diagnosis and treatment of gonadal dysfunction.

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), released by the hypothalamus.[1-3] LH, also called interstitial cell-stimulating hormone (ICSH) in men, is a glycoprotein with a molecular weight of approximately 30,000 daltrons.[4] It is composed of two non-covalently associated dissimilar amino acid chains, alpha and beta.[5] 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.[6-8]

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.[9,10] 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.[4] 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.[11]

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.[3,10] 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.[12] 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.[12]

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. LH secretion is not regulated in these cases.[10,13] 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.[10,14]

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. 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.[10]

In the differential diagnosis of hypothalamic, pituitary, or gonadal dysfunction, assays of LH concentration are routinely performed in conjunction with FSH assays as 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[15,16]. The assay system utilizes a mouse monoclonal anti-α-LH antibody 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 a 45 minute incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of Tetramethylbenzidine (TMB) is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 1N HCl, and the resulting yellow color is measured spectrophotometrically at 450 nm. The concentration of LH is directly proportional to the color intensity of the test sample.

REAGENTS AND MATERIALS PROVIDED

1. *Antibody-Coated Wells (96 wells)*
   Microtiter wells coated with mouse monoclonal anti-α-LH antibody.
2. *Enzyme Conjugate Reagent (13 mL)*
   Contains mouse monoclonal anti-β-LH conjugated to horseradish peroxidase.
3. *Reference Standard Set*
   Contains 0, 5, 15, 50, 100, and 200 mIU/ml (WHO, 1st IRP, 68/40) human LH in bovine serum with preservatives.

Lyophilized. See instructions for reconstitution under Reagent Preparation.

4. *TMB Reagent (11 mL)*
   Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution.
5. *Stop Solution - 1N HCl (11 mL)*
   Contains diluted hydrochloric acid.

Materials Required But Not Provided

1. Distilled or deionaized water
2. Precision pipettes: 0.05, 0.1, 0.2, and 1 ml
3. Disposable pipette tips
4. Microtiter well reader capable of reading absorbance at 450nm
5. Vortex mixer or equivalent
6. Absorbent paper
7. Linear graph paper
8. QC material (e.g., BioRad LyphoCheck Controls)

Warnings and Precautions

1. **CAUTION:** This kit contains human material. The source material used for manufacture of this kit tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling and disposal should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.[21]
2. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
3. Do not use the reagent when it becomes cloudy or contamination is suspected.
4. Do not use the reagent if the vial is damaged.
5. Replace caps on reagents immediately. Do not switch caps.
6. Each well can be used only once.
7. Do not pipette reagents by mouth.
8. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
9. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
10. For in vitro diagnostic use.

Storage Conditions

1. Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. The opened and used reagents are stable until the expiration date if stored properly at 2-8°C.
3. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

Instrumentation

A microtiter well reader with a bandwidth of 10nm or less and an optical density range of 0 to 2 OD or greater at 450nm wavelength is acceptable for absorbance measurement.

Specimen Collection and Preparation

1. 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. Avoid grossly hemolytic, lipemic, or turbid samples.
2. Specimens should be capped and may be stored for up to 48 hours at 2-8°C prior to assaying. Specimens 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.

Reagent Preparation

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Reconstitute each lyophilized standard with 1.0 mL dH_20. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be stored sealed at 2-8°C, and are stable at 2-8°C for at least 30 days.

Procedural Notes

1. Manual Pipetting: It is recommended that no more than 32 wells are used for each assay run. Pipetting of all standards, samples, and controls should be completed within 3 minutes. A multi-channel pipette is recommended.
2. Automated Pipetting: A full plate of 96 wells may be used in each assay run. However, it is recommended that pipetting of all standards, samples, and controls be completed within 3 minutes.
3. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
4. It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

Assay Procedure

1. Secure the desired number of coated wells in the holder.
2. Dispense 50µl of standards, 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.
5. Incubate at room temperature (18-25°C) for 45 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the microtiter wells 5 times with distilled or dionized 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 5 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. ***Ensure that all of the blue color changes completely to yellow.***
13. Read absorbance at 450nm with a microtiter plate reader within 15 minutes.

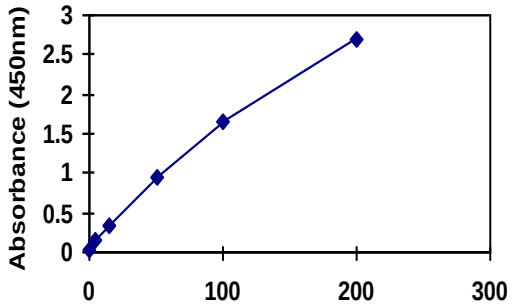
CALCULATION OF RESULTS

1. Calculate the average absorbance value (A_{450}) for each set of reference standards, controls and samples.
2. 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 (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. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. Any diluted samples must be further corrected by the appropriate dilution factor.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with absorbency 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.

LH (mIU/mL)	Absorbance (450 nm)
0	0.043
5	0.148
15	0.328
50	0.947
100	1.656
200	2.704



STANDARDIZATION

The LH Reference Standards are calibrated against the World Health Organization's First International Reference Preparation of Luteinizing Hormone for Immunoassay, (WHO 1st IRP-68/40).

EXPECTED VALUES

Each laboratory should establish its own normal range based on patient population. The information provided below is cited from Reference #16:

Adult	mIU/ml
Male	1.24-7.8
Female	
Follicular phase:	1.68-15
Ovulatory peak:	21.9-56.6
Luteal phase:	0.61-16.3
Postmenopausal:	14.2-52.3

PERFORMANCE CHARACTERISTICS

1. Accuracy

A statistical study using 113 patient samples with LH concentrations ranging from 0.1 to 56 mIU/ml demonstrated good correlation of results with a commercially available kit as shown below. Comparison between this kit and Nichols Institute's Allegro®LH kits provides the following data:

Correlation coefficient = 0.958
Slope = 1.115
Intercept = 0.49
Mean = 10.1 mIU/mL
Nichols Mean = 8.6 mIU/mL

2. Sensitivity

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

3. Precision

a. Intra-Assay Precision

Within-run precision was determined by replicate determinations of three different control sera in one assay.

Serum Sample	1	2	3
Number of Replicates	18	18	18
Mean LH (mIU/ml)	4.9	23.6	57.9
Standard Deviation	0.3	1.4	3.9
Coefficient of Variation (%)	6.5%	5.8%	6.8%

b. Inter-Assay Precision

Between-run precision was determined by replicate measurements of three different control sera over a series of individually calibrated assays.

Serum Sample	1	2	3
Number of Replicates	24	24	24
Mean LH (mIU/ml)	5.1	23.9	57.7
Standard Deviation	0.4	1.7	3.3
Coefficient of Variation (%)	8.1%	7.1%	5.7%

3. Recovery and Linearity Studies

a. Recovery

Patient serum samples of known LH levels were mixed and assayed in duplicate. Average recovery = 102.8%.

Expected Concentration (mIU/ml)	Observed Concentration (mIU/ml)	% Recovery
5.98	6.48	108.4%
23.65	25.31	107.1%
35.64	36.43	102.2%
46.95	51.10	108.8%
72.32	69.37	95.9%
91.78	86.61	94.4%

b. Linearity

Two patient samples were serially diluted with zero mIU/ml standards in a linearity study. The average recovery was 101.2%.

#	Dilution	Expected Conc. (mIU/ml)	Observed Conc. (mIU/ml)	% Recovery
1.	1:32	6.60	6.98	105.8
	1:16	13.19	13.88	105.3
	1:8	26.37	28.59	108.4
	1:4	52.74	54.72	103.8
	1:2	105.47	105.47	100.1
			Average =	***104.7%***
2.	1:64	2.44	2.34	95.9
	1:32	4.88	4.97	101.8
	1:16	9.76	9.34	95.7
	1:8	19.52	18.70	95.8
	1:4	39.04	39.17	100.3
	1:2	78.08	78.08	100.0
			Average =	***98.3%***

5. Specificity

The following hormones were tested for cross-reactivity in the assay:

Hormone Tested	Concentration	Intensity Equiv. to LH (mIU/mL)
HCG	20 mIU/ml	<1
(WHO 1st IRP 75/537)	150 mIU/ml	<1
	1,000 mIU/ml	1.0
	5,000 mIU/ml	4.5
	10,000 mIU/ml	8.9
	25,000 mIU/ml	20.5
	200,000 mIU/ml	41.0
	400,000 mIU/ml	65.0
NOTE: Pregnancy results in elevated levels of hCG, therefore the use of this LH assay is not recommended during pregnancy or immediately post-partum.		
TSH	31.5 uIU/ml	<1
(WHO 2nd IRP 80/558)	62.5 uIU/ml	1.0
	125.0 uIU/ml	1.8
	250 uIU/ml	3.0
	500.0 uIU/ml	5.5
FSH	125 mIU/ml	1.0
(WHO 2nd IRP HMG)	250 mIU/ml	1.9
	500 mIU/ml	3.5

6. Hook Effect

No hook effect is observed in this assay at LH concentrations up to 4,000 mIU/mL.

QUALITY CONTROL

Good laboratory practice requires that quality control specimens (controls) be run with each calibration curve to verify assay performance. Controls containing sodium azide cannot be used. To ensure proper performance, control material should be assayed repeatedly to establish mean values and acceptable ranges.

LIMITATIONS OF THE PROCEDURE

1. 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.
2. 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.
3. The serum samples used in this assay must not contain any additives; EDTA has been found to interfere with the test procedure.
4. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
5. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

REFERENCES

1. Marshall, J.C.: Clinic. In Endocrinol. Metab., 4, 545 (1975).
2. Jeffcoate, S.L.: Clinic. In Endocrinol. Metabl. 4, 521 (1975).
3. Cohen, K.L.: Metabolism, 26, 1165 (1977).
4. Shome, B. and Parlow, A.F.: J. Clin. Endocrinol. Metab., 39, 199 (1974).
5. Lundy, L.E., Lee, S.G., Levy, W., et. al: Obstet. Gynecol., 44, 14 (1974).
6. Ross, F.T., Vande Wiele, R.L. and Franty, A.G.: Text. Of Endocrinol., Chapter 7, Ed: R.H. Williams, W.B. Saunders, Philadelphia (1981).
7. Speroff, L.: Clinic. Gynecol. Endocrinol. and Infert., Chapter 3, Ed: L. Speroff, R.H. Glass and M.G. Kase, Williams & Wilkins, Baltimore (1978).
8. Rebar, R.W., Erickson, G.G. and Yen, S.S.C.: Fertil. Steril., 37, 35 (1982).
9. Catt, K.J. and Pierce, J.G.: Reprod. Endocrinol., Chapter 2, Ed: S.S.C. Yen and R.B. Jaffe, Philadelphia (1978).
10. Leonard, J.M., Leach, R.B., Couture, M. and Paulsen, C.A.: J. Clinic. Endocrinol., 34, 209 (1972).
11. Reiter, E.O. and Lulin, H.E.: J. Clinic. Endocrinol., 33, 957 (1971).
12. Abraham, G.E., Ed.: Radioassay Systems in Clinic. Endocrinol., Marcel Dekker, Inc., New York (1981).
13. Engvall, E., Methods in Enzymology, Volume 70, VanVunakis, H. and Langone, J.J. (eds.), Academic Press, New York, 419 (1980).
14. Uotila, M., Ruoslahti, E. and Engvall, e., J. Immunol. Methods, 42, 11 (1981).
15. USA Center for Disease Control/National Institute of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories", 1984.
16. Clinical Guide to Laboratory Tests. Ed. N.W. Tietz, 3rd Ed., W.B. Saunders Company, Philadelphia, PA 19106, 1995.

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