Atlas Medical CE

LH ELISA

IVD For i*n vitro* diagnostic and professional use only

Store at 2-8 °C

96 Tests

Intended use

Enzyme-linked Immunosorbent for the *in vitro* quantitative determination of luteinizing hormone (LH) in human serum.

Introduction

Luteinizing hormone (LH) is a glycoprotein of 29.5KDa and 121 amino acids produced by gonadotropic cells of the anterior pituitary gland. LH is a heterodimer consisting of one α and one β subunits attached to three sugar chains. LH homeostasis, mediated by the gonadotropin-releasing hormone, is key to proper sexual development and functioning. In women, LH regulates the menstrual cycle in women; it is secreted in pulses into the bloodstream to stimulate the growth and functioning of ovarian follicles and the subsequent production of estrogen and progesterone. The highest LH concentrations occur during the mid-cycle peak and induce ovulation and formation of the corpus luteum, the principal secretion product of which is progesterone. In the Leydig cells of the testes, LH stimulates the production of testosterone. Determination of the LH concentration is used in the elucidation of dysfunctions within the hypothalamus-pituitary-gonads system. The determination of LH in conjunction with FSH is utilized for the following indications: congenital diseases with chromosome aberrations (e.g. Turner's syndrome), polycystic ovaries (PCO), clarifying the causes of amenorrhea, menopausal syndrome, and suspected Leydig cell insufficiency.

Test Principle

This assay is based on the sandwich format; monoclonal anti-LH (pre-coated on microwells) and enzyme-labelled anti-LH antibodies simultaneously bind LH present in the sample forming an immobilized sandwich complex. Washing removes any unbound material. The substrate is then added and is catalyzed by the HRP enzyme in this complex to induce a chromogenic reaction. The intensity of the resulting color is proportional to the amount of LH in the sample. This is measured as absorbance at 450 and 620-630 nm.

Materials

Materials provided

1. **Coated Microplate**
   One plate 8x12 strips, pre-coated with mouse monoclonal Anti-LH.
2. **Calibrators**
   Six white cap vials, 1 ml each, ready to use.

Calibrator	LH Concentration (mIU/ml)
A	0
B	5
C	20
D	50
E	100
F	200

3. **Enzyme Conjugate**
   One red cap vial, 11 ml of HRP (horseradish peroxidase) labeled mouse monoclonal Anti-LH in Tris-NaCl buffer containing BSA (bovine serum albumin). Contains 0.1%ProClin300® preservative.
4. **Substrate Solution**
   One brown cap vial, 11ml, ready to use, (tetramethylbenzidine) TMB.
5. **Stop Solution**
   One yellow cap vial, 6.0 ml of 1 mol/l sulfuric acid.
6. **Wash Solution Concentrate**
   One transparent cap bottle, 25 ml (40X concentrated), PBS-Tween wash solution.
7. **Package insert.**
8. **1 piece of plate cover.**

Materials required but not provided

1. Microplate reader with 450nm and 620nm wavelength absorbent capability.
2. Microplate washer.
3. Incubator.
4. Plate shaker.
5. Micropipettes and multichannel micropipettes
6. Absorbent paper.
7. Distilled water

Packaging Contents

REF **8.10.04.0.0096**

Contains reagents enough to make 96 tests.

Reagent Storage and Stability

1. Store at 2-8°C.
2. Seal and return all the other unused reagents to 2-8 °C, under which conditions the stability will be retained for 2 months, or until the labeled expiry date, whichever is earlier.

Precautions and Warnings

1. For *in vitro* diagnostic use only. For professional use only.
2. Exercise the normal precautions required for handling all laboratory reagents.
3. Disposal of all waste material should be in accordance with local guidelines.
4. Do not use reagents beyond the labeled expiry date.
5. Do not mix or use components from kits with different batch codes.
6. All the specimen and reaction wastes should be considered potentially biohazard. The handling of specimens and reaction wastes should be in accordance with the local regulations and guidelines.
7. The Stop Solution contains sulfuric acid, which can cause severe burns. In the event of contact with eyes, rinse immediately with plenty of water and seek medical advice.
8. Neutralized acids and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. Exposure to 1.0% sodium hypochlorite for 30 minutes may be necessary to ensure effective decontamination.
9. Some reagents contain 0.05% or 0.1% ProClin 300 which may cause sensitization by skin contact, which must therefore be avoided. Reagents and their containers must be
10. Substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
11. Do not smoke, drink, eat or apply cosmetics in the work area.
12. Do not pipette by mouth. Wear protective clothing, disposable gloves and eye/face protection when handling samples and reagents. Wash hands after use.
13. If any of the reagents comes into contact with the skin or eyes, wash the area extensively with water.

Collection, Handling and Preparation of Specimen

- Human serum is recommended for this assay.
- Cap and store the samples at 18-25 ℃ for no more than 8 hours. Stable for 7 days at 2-8℃, and 1 month at -20℃. Freeze only once.
- Do not use heat-inactivated samples.

- Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation.
- Avoid grossly hemolytic, lipemic or turbid samples.

Reagent Preparation

Add deionized water to the 40X concentrated wash solution concentrate. Dilute 25 ml of wash solution concentrate with 975 ml of deionized water to a final volume of 1000 ml stable for 2 months at room temperature.

Equilibrate kit and samples to room temperature (18-25°C) before use and mix gently.

Procedure

1. Use only the number of wells required and format the microplates' wells for each calibrator and sample to be assayed.
2. Add **25 µL of calibrators or samples** to each well.
3. Add **100 µL of enzyme conjugate** to each well.
4. Shake the microplate gently for 30 seconds to mix.
5. Cover the plate with a plate lid and incubate at **37 °C for 60 minutes.**
6. Discard the contents of the micro plate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add **350 µL of diluted wash solution**, decant (tap and blot) or aspirate. Repeat 4 additional times for a total of 5 washes. An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
8. Add **100 µL of substrate** to each well.
9. Incubate at ambient temperature (18-25℃) in the dark for reaction for 20 minutes. Do not shake the plate after substrate addition.
10. Add **50 µL of stop solution** to each well.
11. Shake for 15-20 seconds to mix the liquid within the wells. It is important to ensure that the blue color changes to yellow completely.
12. Read the absorbance of each well at 450 nm (using 620 to 630 nm as the reference wavelength to minimize background) in a micro plate reader. Results should be read within 30 minutes of adding the stop solution.

Interpretation of the results

1. Record the absorbance obtained from the printout of the microplate reader.
2. Calculate the mean absorbance of any duplicate measurements and use the mean for the following calculation.
3. Plot the common logarithm of absorbance against concentration in mIU/ml for each calibrator.
4. Draw the best-fit curve through the plotted points on linear graph paper. Point-to-Point method is suggested to generate a calibration curve.

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

Sample	Value (mIU/ml)	Absorbance
Cal A	0	0.015
Cal B	5	0.155
Cal C	20	0.518
Cal D	50	1.15
Cal E	100	2.19
Cal F	200	3.368
Ctrl 1	16.7	0.438
Ctrl 2	81.11	1.797
Sample	24.7	0.617

Expected values

Men:

1.5 - 9.6 mIU/mL

Women:

- Follicular phase: 2.1 - 12.7 mIU/mL
- Ovulation phase: 14 - 105 mIU/mL
- Luteal phase: 1 - 12 mIU/mL
- Postmenopause: 7.5 - 65 mIU/mL

Studies with the Atlas LH assay have revealed the following LH values:

Test subjects	N	LH (mIU/mL)		
		Percentile		
		50th	5th	95th
Men	404	4.4	1.7	9.1
Women				
Follicular phase	339	6.1	2.5	11.7
Ovulation phase	145	29.8	17	98
Luteal phase	416	4.9	3	11
Postmenopause	209	28.6	12	59

LH/FSH quotient: Quotients have been calculated from the results obtained with the Atlas LH assay and the Atlas FSH assay in the samples of healthy women of child-bearing age.

The following medians have been calculated:

Follicular phase: 0.82 (n = 331)

Luteal phase: 1.12 (n = 306)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Quality control

Each laboratory should assay controls at levels in the low, normal, and elevated range for monitoring assay performance. Controls should be treated as unknowns and values determined in every test procedure performed. The recommended controls requirement for this assay are to purchase trueness control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the concentration ranges printed on the labels.

Limitations of the Test

1. Criterion: Recovery within ± 10 % of initial value.
2. Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.
3. Samples of neonates have not been tested with the Elecsys LH assay.
4. In rare cases, interference due to extremely high titers of antibodies to analyte specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.
5. For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

0.200-200 mIU/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as <0.200 mIU/mL. Values above the measuring range are reported as >200 mIU/mL.

Lower limits of measurement

Lower detection limit

Lower detection limit: 0.200mIU/mL

The detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

Performance Characteristics

Representative performance data are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Atlas reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 2 times daily for 20 days (n = 40). The following results were obtained:

		Repeatability*		Intermediate precision	
Sample	Mean mIU/mL	SD mIU/mL	CV %	SD mIU/mL	CV %
Human Serum 1	4.85	0.397	8.18	0.381	7.85
Human Serum 2	23.93	1.742	7.28	1.996	8.34
Human Serum 3	59.21	3.322	5.61	3.677	6.21
PC Universal 1	12.85	0.892	6.94	0.961	7.48
PC Universal 2	48.39	2.671	5.52	2.826	5.84

*Repeatability = within-run precision

Method comparison

A comparison of the Atlas LH assay (y) with the Roche Cobas LH (x) using clinical samples gave the following correlations: Number of samples measured: 121

Linear regression

y = 1.0643x + 0.106

r = 0.9798

The sample concentrations were between approx. 0 and 191 mIU/mL.

Analytical specificity

For the monoclonal antibodies used, the following cross-reactivities were found:

FSH, TSH, hCG, hGH, hPL < 0.1 %.

Functional sensitivity

0.210mIU/mL

The functional sensitivity is the analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20%.

Hook Effect

There is no high-dose hook effect at LH concentrations up to 1150mIU/mL

Interference

1. The assay is unaffected by icterus (bilirubin <1129 µ mol/L or <66 mg/dL), hemolysis (Hb <0.621 mmol/L or <1 g/dL), lipemia (Intralipid <1900 mg/dL) and biotin (<205 nmol/L or <50 ng/mL).
2. No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL.

Literature References

1. Johnson MR, Carter G, Grint C, et al. Relationship between ovarian steroids, gonadotropin and relaxin during the menstrual cycle. Acta Endocrinol 1983;129/2:121-125.
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3. Runnebaum B, Rabe T. Gynäkologische Endokrinologie und Fortpflanzungsmedizin Springer Verlag 1994. Band 1:17,253-255, Band 2:152-154,360,348. ISBN 3-540-57345-3, ISBN 3-540-57347-X.
4. Schmidt-Mathiesen H. Gynäkologie und Geburtshilfe. Schattauer Verlag 1992.Scott MG, Ladenson JH, Green ED, et al. Hormonal evaluation of female infertility and reproductive disorders. Clin Chem 1989;35:620-630.

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PPI1433A01
Rev B (10.03.2022)

REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry