LH

Luteinizing Hormone (ELISA)

diasino

REF: DS177709 Σ 96

Intended use
The DiaSino LH assay is an enzyme-linked immunosorbent assay (ELISA) for the in vitro quantitative determination of luteinizing hormone (LH) in human serum. For professional use only.

Summary
LH (luteinizing hormone), together with FSH (follicle stimulating hormone), belongs to the gonadotropin family. LH and FSH regulate and stimulate the growth and function of the gonads (ovaries and testes) synergistically. Like FSH, TSH and hCG, LH is a glycoprotein consisting of two subunits (α- and β-chains).[1,2,3] This proteohormone, which consists of 121 amino acids and three sugar chains, has a molecular weight of 29500 daltons. In women, the gonadotropins act within the hypothalamus-pituitary-ovary regulating circuit to control the menstrual cycle.[4] LH and FSH are released in pulses from the gonadotropic cells of the anterior pituitary and pass via the bloodstream to the ovaries. Here the gonadotropins stimulate the growth and maturation of the follicle and hence the biosynthesis of estrogens and progesterones. 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.[1,4]

Test principle
Sandwich principle. Total duration of assay: **80 minutes**.
- Sample, Anti-LH coated microwells and enzyme labeled Anti-LH are combined.
- During the incubation, LH presents in the sample is allowed to react simultaneously with the two antibodies, resulting in the LH molecules being sandwiched between the solid phase and enzyme-linked antibodies.
- After washing, a complex is generated between the solid phase, the LH within the sample and enzyme-linked antibodies by immunological reactions.
- Substrate solution is then added and catalyzed by this complex, resulting in a chromogenic reaction. The resulting chromogenic reaction is measured as absorbance.
- The absorbance is proportional to the amount of LH in the sample.

Reagents
Materials provided
- **LH Coated Microplate - symbol** LH PLATE 8 x 12 strips, 96 wells, pre-coated with mouse monoclonal Anti-LH.
- **LH Calibrators - symbols** LH CAL A-F 6 vials, 1 mL each, ready to use; Concentrations: 0(**A**), 5(**B**), 20(**C**), 50(**D**), 100(**E**) and 200(**F**) mIU/mL.
- **LH Enzyme Conjugate - symbol** LH CONJ 1 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.
- **Substrate - symbol** SUBSTRATE 1 vial, 11mL, ready to use, (tetramethylbenzidine) TMB.
- **Stop Solution - symbol** STOP 1 vial, 6.0 mL of 1 mol/L sulfuric acid.
- **Wash Solution Concentrate - symbol** WASH 40X 1 vial, 25 mL (40X concentrated), PBS-Tween wash solution.
- **IFU -** 1 copy.
- **Plate Lid**: 1 piece.

Materials required (but not provided)
- Microplate reader with 450nm and 620nm wavelength absorbent capability.
- Microplate washer.
- Incubator.
- Plate shaker.
- Micropipettes and multichannel micropipettes delivering 50µl with a precision of better than 1.5%.
- Absorbent paper.
- Distilled water

Precautions and warnings
- For in vitro diagnostic use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Disposal of all waste material should be in accordance with local guidelines.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different batch codes.
- 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.
- 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.
- 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.
- 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 disposed of safely. If swallowed, seek medical advice immediately and show this container or label.
- 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.
- For information on hazardous substances included in the kit please refer to the Materials Safety Data Sheet (MSDS), which is available on request.
- Do not smoke, drink, eat or apply cosmetics in the work area.
- Do not pipette by mouth. Wear protective clothing, disposable gloves and eye/face protection when handling samples and reagents. Wash hands after use.
- If any of the reagents comes into contact with the skin or eyes, wash the area extensively with water.

Incident report
Any suspected serious incidents related to this assay shall be immediately reported to DiaSino, DiaSino's Authorized Representative in the EU, and the national competent authorities of the Member States where the users and/or patients are located.

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

Specimen collection and preparation
- 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.

Calibration
The DiaSino LH ELISA has been standardized against the WHO 2nd IS 80/552.
Recalibration is recommended when a new reagent lot is used, or the quality controls are out of specified range.

Quality control
Each laboratory should have assay controls at levels in the low, normal, and elevated range for monitoring assay performance. The 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.

Wash solution (40X dilution)
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.

Test procedure
Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25 ℃) before measurement. Mix all reagents through gently inverting prior to use.
- Use only the number of wells required and format the microplates' wells for each calibrator and sample to be assayed.
- Add **25 µL** of **calibrators** or **samples** to each well.
- Add **100 µL** of **enzyme conjugate** to each well.
- Shake the **microplate** gently for **30 seconds** to mix.
- Cover the plate with a plate lid and incubate at **37 ℃** for 6**0 minutes**.
- Discard the contents of the micro plate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
- Add **350 µL** of **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.
- Add **100 µL** of **substrate** to each well.
- Cover and **Incubate** at **ambient temperature (18-25℃)** in the dark for reaction for **20 minutes**. Do not shake the plate after substate addition.
- Add **50 µL** of **stop solution** to each well.
- 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.
- Read the absorbance of each well at **450 nm** (using 620 to 630 nm as the reference wavelength to minimize well imperfections) in a micro plate reader. The results should be read within **30 minutes** of adding the stop solution.

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

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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
Calibrator A	0	0.015
Calibrator B	5	0.155
Calibrator C	20	0.518
Calibrator D	50	1.15
Calibrator E	100	2.19
Calibrator F	200	3.368
Control 1	16.7	0.438
Control 2	81.11	1.797
Sample	24.7	0.617

Limitations - interference
- The assay is unaffected by icterus (bilirubin < 1129 µmol/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).
- Criterion: Recovery within ± 10 % of initial value.
- 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.
- No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL.
- 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.
- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

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 detection limit
0.200 mIU/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).

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 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	399	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 DiaSino LH assay and the DiaSino 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.

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

Precision
Precision was determined using 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 LH assay (y) with the Elecsys LH (x) using 97 clinical samples gave the following correlations:
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
0.210 mIU/mL
The functional sensitivity is the lowest 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 1150 mIU/mL.

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.
2. Beastall GH, Ferguson KM, O'Reilly DSJ, et al. Assays for follicle stimulating hormone and luteinizing hormone: Guidelines for the provision of a clinical biochemistry service. Ann Clin Biochem 1987;24:246-262.
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.

Symbols

IVD **In vitro diagnostic medical device**	8°C 2°C **Temperature limit**	**Consult instructions for use**	REF **Catalog number**
LOT **Batch code**	**Date of manufacture**	**Use-by date**	Σ **Contains sufficient for <n> tests**
Manufacturer	**Do not use if package is damaged and consult instructions for use**	CE **European Conformity**	EC REP **Authorized representative in the European Community**

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