LH

cobas®

Luteinizing hormone

REF	Σ	SYSTEM
11732234 122	100	Elecsys 2010 MODULAR ANALYTICS E170 **cobas e** 411 **cobas e** 601 **cobas e** 602

English

Intended use

Immunoassay for the in vitro quantitative determination of luteinizing hormone in human serum and plasma.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

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.[1,2,3]

Like FSH, TSH and hCG, LH is a glycoprotein consisting of two subunits (α- and β-chains). This proteohormone, which consists of 121 amino acids[2] and three sugar chains, has a molecular weight of 29500 daltons.[3]

In women, the gonadotropins act within the hypothalamus-pituitary-ovary regulating circuit to control the menstrual cycle.[4,5]

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.[1,4,5]

The Elecsys LH assay employs two monoclonal antibodies specifically directed against human LH. The two specific antibodies used recognize particular conformations, with the biotinylated antibodies detecting an epitope constructed from both subunits whereas the antibody with the ruthenium complex[a)] label detects an epitope from the β-subunit. As a result, the Elecsys LH assay shows negligible cross-reactivity with FSH, TSH, hCG, hGH, and hPL.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 20 µL of sample, a biotinylated monoclonal LH-specific antibody, and a monoclonal LH-specific antibody labeled with a ruthenium complex form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

Reagents - working solutions

The reagent rackpack is labeled as LH.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-LH-Ab~biotin (gray cap), 1 bottle, 10 mL:
Biotinylated monoclonal anti-LH antibody (mouse) 2.0 mg/L; TRIS buffer 50 mmol/L, pH 8.0; preservative.

R2 Anti-LH-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 10 mL:
Monoclonal anti-LH antibody (mouse) labeled with ruthenium complex 0.3 mg/L; TRIS buffer 50 mmol/L, pH 8.0; preservative.

Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines.

Safety data sheet available for professional user on request.

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in from the respective reagent barcodes.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	12 weeks
on the analyzers	8 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-, Na-, NH_4^+-heparin, K_3-EDTA and sodium fluoride/potassium oxalate plasma. When sodium citrate is used, the results must be corrected by + 10 %.

Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within < ± 2x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Stable for 14 days at 2-8 °C, 6 months at -20 °C. Freeze only once.[6]

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems

from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 03561097190, LH CalSet II, for 4 x 1 mL
- REF 11731416190, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2
- REF 11731416160, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2 (for USA)
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for Elecsys 2010 and **cobas e** 411 analyzers:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- REF 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500316, Elecsys SysClean, 5 x 100 mL system cleaning solution
- REF 11298500160, Elecsys SysClean, 5 x 100 mL system cleaning solution (for USA)

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

Bring the cooled reagents to approx. 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Calibration

Traceability: This method has been standardized against the 2nd International Standard (NIBSC) 80/552.

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Universal.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in mIU/mL or IU/L).

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.

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

In vitro tests were performed on 17 commonly used pharmaceuticals. No interference with the assay was found.

Samples of neonates have not been tested with the Elecsys LH assay.

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.

Limits and ranges

Measuring range

0.100-200 mIU/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.100 mIU/mL. Values above the measuring range are reported as > 200 mIU/mL.

Lower limits of measurement

LH

Luteinizing hormone

Lower detection limit of the test

Lower detection limit: 0.100 mIU/mL

The lower detection limit represents the lowest measurable 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).

Dilution

Not necessary due to the broad measuring range.

Expected values

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

Test subjects[b)]	N	LH mIU/mL		
		Percentile		
		50th	5th	95th
Men	322	4.0	1.7	8.6
Women				
• Follicular phase	316	5.9	2.4	12.6
• Ovulation phase	56	30.8	14.0	95.6
• Luteal phase	280	4.3	1.0	11.4
• Postmenopause	132	29.1	7.7	58.5

b) Reference ranges for children are available on request and are also contained in the Elecsys LH product information.

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

Follicular phase: 0.82 (n = 315)

Luteal phase: 1.12 (n = 279)

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 on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 6 times daily for 10 days (n = 60); repeatability on MODULAR ANALYTICS E170 analyzer, n = 21. The following results were obtained:

Elecsys 2010 and **cobas e** 411 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean mIU/mL	SD mIU/mL	CV %	SD mIU/mL	CV %
Human serum 1	0.54	0.01	1.8	0.03	5.2
Human serum 2	27.2	0.21	0.8	0.54	2.0
Human serum 3	50.7	0.41	0.8	1.01	2.0
PC[c)] Universal 1	9.38	0.11	1.1	0.19	2.0
PC Universal 2	44.8	0.42	0.9	0.83	1.9

c) PC = PreciControl

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers					
	Repeatability			**Intermediate precision**	
Sample	Mean mIU/mL	SD mIU/mL	CV %	Mean mIU/mL	SD mIU/mL	CV %
Human serum 1	6.15	0.08	1.2	5.81	0.12	2.0
Human serum 2	92.2	0.68	0.7	89.1	1.47	1.6
Human serum 3	164	1.41	0.9	159	3.47	2.2
PC Universal 1	6.67	0.05	0.8	6.63	0.14	2.1
PC Universal 2	54.6	0.35	0.6	54.2	1.13	2.1

Method comparison

A comparison of the Elecsys LH assay (y) with the Enzymun-Test LH method (x) using clinical samples gave the following correlations:

Number of samples measured: 166

Passing/Bablok[7]	Linear regression
y = 1.09x - 0.46	y = 1.14x - 0.80
τ = 0.929	r = 0.993

The sample concentrations were between approx. 1.3 and 123 mIU/mL.

Analytical specificity

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

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

References

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2 Collip JB. William Henry Welch lectures: Some recent advances in physiology of anterior pituitary J. MA Sinai Hosp 1934;1:28-71.

3 Johnson MR, Carter G, Grint C, et al. Relationship between ovarian steroids, gonadotropin and relating during the menstrual cycle. Acta Endocrinol 1993;129/2:121-125.

4 Runnebaum B, Rabe T. Gynäkologische Endokrinologie und Fortpflanzungsmedizin Springer Verlag 1994. Band 1:17,202-205,252-253, Band 2:350,360-362. ISBN 3-540-57345-3, ISBN 3-540-57347-x.

5 Scott MG, Ladenson JH, Green ED, et al. Hormonal evaluation of female infertility and reproductive disorders. Clin Chem 1989;35:620-630.

6 Tietz NW. Clinical Guide To Laboratory Tests. 3rd ed. Philadelphia, Pa: WB Saunders Co, 1995:410.

7 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT Contents of kit

LH

Luteinizing hormone

cobas®

SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing

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