Elecsys LH

cobas®

Materials provided

REF		Σ	SYSTEM
09755721190	09755721501	100	**cobas** e 402 **cobas** e 801

For reagents, refer to the "Reagents" section.

Materials required (but not provided)

REF	Description
09557423190	LH CalSet II, for 4 × 1.0 mL
11731416160	PreciControl Universal, for 4 × 3.0 mL
	General laboratory equipment
	cobas e analyzer

Additional materials for **cobas** e 402 and **cobas** e 801 analyzers:

REF	Description
06908799190	ProCell II M, 2 × 2 L system solution
04880293190	CleanCell M, 2 × 2 L measuring cell cleaning solution
07485409001	Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
06908853190	PreClean II M, 2 × 2 L wash solution
05694302001	AssayTip/AssayCup tray, 6 magazines × 6 magazine stacks × 105 assay tips and 105 assay cups, 3 wasteliners
07485425001	Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution / Elecsys SysClean for Liquid Flow Cleaning Detection Unit
07485433001	PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution / Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
11298500160	ISE Cleaning Solution / Elecsys SysClean, 5 × 100 mL system cleaning solution

For use in the USA only

System information

Short name	ACN (application code number)
LH	10113

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 **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]

Like FSH, TSH and hCG, LH is a glycoprotein dimer consisting of 2 glycosylated noncovalently-linked subunits designated α and β. The α subunit is composed of 92 amino acids and is encoded on the long arm of chromosome 6. The β subunit is composed of 121 amino acids.[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.[1]

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.[6,7]

Elecsys LH

The Elecsys LH assay employs 2 monoclonal antibodies specifically directed against human LH. The 2 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.

- First incubation: 12 µL of sample, a biotinylated monoclonal LH-specific antibody, and a monoclonal LH-specific antibody labeled with a ruthenium complex form a sandwich complex.
- Second incubation: After streptavidin-coated microparticles have been added, 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 II 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 that is instrument-specifically generated by 2-point calibration and a leading calibration curve provided via **cobas** link.

Reagents

The **cobas** e pack is labeled as LH.

M	Streptavidin-coated microparticles, 1 bottle, 6.4 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
R1	Anti-LH-Ab~biotin, 1 bottle, 8.6 mL: Biotinylated monoclonal anti-LH antibody (mouse) 2.0 mg/L; TRIS buffer 50 mmol/L, pH 8.0; preservative.
R2	Anti-LH-Ab~$Ru(bpy)_3^{2+}$, 1 bottle, 8.6 mL: Monoclonal anti-LH antibody (mouse) labeled with ruthenium complex 0.3 mg/L; TRIS buffer 50 mmol/L, pH 8.0; preservative.

Warnings and precautions

For in vitro diagnostic use for healthcare professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste

Warning: Handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards

Apply all relevant local disposal regulations to determine safe disposal.

The Safety Data Sheet is available for professional users on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317	May cause an allergic skin reaction.

Prevention:

P261	Avoid breathing mist or vapours.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves.

Response:

P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Hazardous components:

- 2-methyl-2H-isothiazol-3-one hydrochloride

Product safety labeling follows EU GHS guidance.

Contact phone: +1-800-428-2336

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

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas** e pack **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
on the analyzers	16 weeks

Calibration

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

The predefined leading calibration curve is adapted to the analyzer using the relevant calibrators.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e., not more than 24 hours after the **cobas** e pack was registered on the analyzer).

The calibration interval may be extended based on acceptable calibration verification values determined by the laboratory.

Renewed calibration is recommended as follows:

- every 12 weeks when using the same reagent lot
- every 4 weeks when using the same **cobas** e pack on the analyzer
- as required, such as when quality control findings are outside the defined limits

Quality control

For routine quality control procedures, use PreciControl Universal or other suitable controls.

It is recommended to run the controls for the various concentration ranges individually at least once every 24 hours when the test is in use, once per **cobas** e pack, and following each calibration.

Adjust the limits and control intervals based on the laboratory's individual requirements. If values fall outside the limits, each laboratory is advised to establish corrective measures.

If necessary, repeat sample measurement.

Follow the applicable government regulations and local guidelines.

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

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

Li-heparin, K2 EDTA, and K3 EDTA plasma.

Criterion: slope 0.9-1.1 + intercept within ≤ ± 0.3 mIU/mL + coefficient of correlation ≥ 0.95.

Stable for 5 days at 20-25 °C, 14 days at 2-8 °C, 6 months at -20 °C (± 5 °C).

Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing. 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 and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, analyze and measure samples, calibrators, and controls on the analyzers within 2 hours.

Sample stability claims were established based on experimental data by the manufacturer or on reference literature, and only for the temperatures / time frames as stated in the Method Sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Test procedure

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 available via **cobas** link.

For optimum performance of the assay, follow the instructions given in this document for the corresponding analyzer. For analyzer-specific assay instructions, refer to the corresponding User Guide.

Resuspension of the microparticles takes place automatically prior to use.

Place the cooled (stored at 2-8 °C) **cobas** e pack on the reagent manager.

Avoid foam formation.

The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas** e pack.

Calculation

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

Limitations and interferences

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations, and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1129 µmol/L or ≤ 66 mg/dL
Hemoglobin	≤ 0.621 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 1900 mg/dL
Biotin	≤ 205 nmol/L or ≤ 50 ng/mL
Rheumatoid factors	≤ 1200 IU/mL

Criterion: For concentrations from 0.3-20 mIU/mL the deviation is ± 2.5 mIU/mL. For concentrations from 20-200 mIU/mL the deviation is ± 10 %.

It is advised not to take samples from patients receiving therapy with high biotin doses (i.e., > 5 mg/day) until at least 8 hours following the last biotin administration.

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

Pharmaceutical substances

In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

In rare cases, interference may be caused by extremely high titers of antibodies to analyte-specific antibodies, streptavidin, or ruthenium. These effects are minimized by suitable test design.

For diagnostic purposes, always assess the results in conjunction with the patient's medical history, clinical examination, and other findings.

Limits and ranges

Measuring range

0.3-200 mIU/mL (defined by the Limit of Detection and the maximum of the leading calibration curve).
Values below the Limit of Detection are reported as < 0.3 mIU/mL. Values above the measuring range are reported as > 200 mIU/mL.

Lower limits of measurement

Limit of Blank, Limit of Detection, and Limit of Quantitation

Limit of Blank = 0.1 mIU/mL

Limit of Detection = 0.3 mIU/mL

Limit of Quantitation = 1 mIU/mL

The Limit of Blank, the Limit of Detection, and the Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th-percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low-concentration samples. The Limit of Detection corresponds to the lowest analyte concentration that can be detected (value above the Limit of Blank with a probability of 95 %).

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

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	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

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 is advised to investigate the transferability of the expected values to its own patient population and, if necessary, to determine its own reference ranges.

Specific performance data

Representative performance data on the analyzer is given below. Results obtained in individual laboratories may differ from the representative performance data.

Analytical specificity

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

Substance	**Additive concentration mIU/mL**	**Cross-reactivity %**
FSH	5000	0.005
TSH	5000	not detectable
hCG	5000	0.003
hGH	2000	not detectable
hPL	5000	not detectable

Precision

Precision was determined using Elecsys reagents, pooled human sera, and controls based on a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day, in duplicate each, for 21 days (n = 84). The following results were obtained:

cobas e 402 and cobas e 801 analyzers					
		Repeatability		**Intermediate precision**	
Sample	**Mean mIU/mL**	**SD mIU/mL**	**CV %**	**SD mIU/mL**	**CV %**
Human serum 1	0.992	0.021	2.2	0.023	2.3
Human serum 2	11.4	0.120	1.0	0.158	1.4
Human serum 3	63.4	0.631	1.0	0.707	1.1
Human serum 4	113	1.20	1.1	1.50	1.3

cobas e 402 and cobas e 801 analyzers					
		Repeatability		Intermediate precision	
Sample	Mean mIU/mL	SD mIU/mL	CV %	SD mIU/mL	CV %
Human serum 5	194	1.80	0.9	2.30	1.2
PreciControl Universal 1	10.7	0.120	1.1	0.177	1.6
PreciControl Universal 2	51.4	0.655	1.3	1.08	2.1

Method comparison

A comparison of the Elecsys LH assay, REF 07027575190 (**cobas** e 801 analyzer; y), with the Elecsys LH assay, REF 11732234122 (**cobas** e 601 analyzer; x), gave the following correlation (mIU/mL):

Number of samples measured: 146

Passing/Bablok[8]	Linear regression
y = 1.058x - 0.0887	y = 1.043x + 0.228
τ = 0.992	r = 1.000

The sample concentrations were between 0.617 and 190 mIU/mL.

Additional information

Additions, deletions, or changes are indicated by a change bar in the margin.

For further information, refer to the User Guide for the corresponding analyzer, to the corresponding application sheets, and to the Method Sheets of all necessary components.

Report any serious incident that has occurred in relation to the device to the manufacturer and the competent authority of the member state in which the user and/or patient is established.

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product meets the specifications stated in the labeling when used in accordance with the labeling and is free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL, OR CONSEQUENTIAL DAMAGES.

Symbols

For definition of symbols used, refer to navifyportal.roche.com.

In addition to the ISO 15223-1 standard, Roche Diagnostics uses the following symbols and signs:

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

References

1 Franchimont P. Regulation of gonadal androgen secretion Horm Res 1983;18(1-3):7-17.

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

3 Armand MK, Crister JK. Reproductive Tissue Banking: Scientific Principles - 186.

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 Howell SJ, Radford JA, Adams JE, et al. Randomized placebo-controlled trial of testosterone replacement in men with mild Leydig cell insufficiency following cytotoxic chemotherapy. Clin Endocrinol (Oxf). 2001;55(3):315-24.

7 Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Fertil Steril. 2004;81(1):19-25.

8 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 USA: Rx only

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany
www.roche.com
+800 5505 6606

Roche Diagnostics
9115 Hague Road
Indianapolis, IN 46256, USA
+1 800 428 2336