Elecsys IL-6

cobas®

REF		Σ	SYSTEM
09015612190	09015612500	100	**cobas e** 402 **cobas e** 801

English

System information

Short name	ACN (application code number)
IL6	10085

Intended use

Immunoassay for the in vitro quantitative determination of Interleukin-6 (IL-6) in serum and plasma. This assay can be used to aid in the management of critically ill patients as early indicator for acute inflammation.

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

Summary

Interleukin-6 (IL-6) is a pleiotropic cytokine with a wide range of functions. It was first described as interferon-β2, plasmacytoma growth factor, and hepatocyte stimulating factor. Later on it was described as human B-cell-stimulating factor 2 (BSF2). In 1988 it was proposed to name it IL-6 as further studies had demonstrated that the protein shows activities not only on B-cells but also on T-cells, hematopoietic stem cells, hepatocytes and brain cells.[1] IL-6 is produced from a single gene encoding a product of 212 amino acids, which is cleaved at the N-terminus to produce a 184 amino acid peptide with a molecular weight between 22-27 kDa.[2] In 1989 it was reported that also immunoreactive complexes in the range of 60-70 kDa were detected in human body fluids in patients with acute bacterial infections.[3]

IL-6 production is rapidly induced in the course of acute inflammatory reactions associated with injury, trauma, stress, infection, brain death, neoplasia, and other situations.[2]

IL-6 concentrations in trauma patients may predict later complications from additional surgical stress or indicate missed injuries or complications.[4,5]

Sequential measurements of IL-6 in serum or plasma of patients admitted to the ICU (intensive care unit) showed to be useful in evaluating the severity of SIRS (systemic inflammatory response syndrome), sepsis and septic shock and to predict the outcome of these patients.[6,7,8] IL-6 is also useful as an early alarm marker for the detection of neonatal sepsis.[9,10,11,12] IL-6 also plays a role in chronic inflammation e.g. rheumatoid arthritis.[13,14]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 18 µL of sample are incubated with a biotinylated monoclonal IL-6-specific antibody.
- 2nd incubation: After addition of a monoclonal IL-6-specific antibody labeled with a ruthenium complex[a)] and streptavidin-coated microparticles, the antibodies form a sandwich complex with the antigen of the sample.
- 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 which is instrument-specifically generated by 2-point calibration and a master curve provided via the **cobas** link.

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

Reagents - working solutions

The **cobas e** pack is labeled as IL6.

M Streptavidin-coated microparticles, 1 bottle, 5.8 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-IL-6-Ab~biotin, 1 bottle, 9.9 mL:
Biotinylated monoclonal anti-IL-6 antibody (mouse) 0.9 µg/mL; phosphate buffer 95 mmol/L, pH 7.3; preservative.

R2 Anti-IL-6-Ab~$Ru(bpy)_3^{2+}$, 1 bottle, 7.6 mL:
Monoclonal anti-IL-6 antibody (mouse) labeled with ruthenium complex 1.5 µg/mL; phosphate buffer 95 mmol/L, pH 7.3; preservative.

Precautions and warnings

For in vitro diagnostic use for health care 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 the safe disposal.

Safety data sheet available for professional user 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.
H412	Harmful to aquatic life with long lasting effects.

Prevention:

P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P273	Avoid release to the environment.
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.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

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 available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

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

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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-heparin, K_2-EDTA and K_3-EDTA plasma.

Plasma tubes containing separating gel can be used.

Criterion: Slope 0.9-1.1 + intercept within ≤ ± 3 pg/mL + coefficient of correlation ≥ 0.95.

Stable for 6 hours at 20-25 °C, 2 days at 2-8 °C, 24 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, 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 and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators 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 05109469190, IL-6 CalSet, for 4 x 2.0 mL
- REF 05341787190, PreciControl Multimarker, for 6 x 2.0 mL
- REF 07299010190, Diluent MultiAssay, 45.2 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

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

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

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.

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.

Calibration

Traceability: This method has been standardized against the NIBSC (National Institute for Biological Standards and Control) 1st IS 89/548 Standard.

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 **cobas e** pack was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Multimarker.

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 **cobas e** pack, 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.

If necessary, repeat the measurement of the samples concerned.

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

Calculation

The analyzer automatically calculates the analyte concentration of each sample in pg/mL.

Limitations - interference

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	≤ 684 µmol/L or ≤ 40 mg/dL
Hemoglobin	≤ 0.621 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 1500 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	≤ 1200 IU/mL

Criterion: For concentrations of 1.5-25 pg/mL the deviation is ≤ 4 pg/mL. For concentrations > 25 pg/mL the deviation is ≤ 15 %.

There is no high-dose hook effect at IL-6 concentrations up to 200000 pg/mL.

Pharmaceutical substances

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

In addition, the following special therapeutic drugs were tested. No interference with the assay was found.

Special therapeutic drugs

Drug	Concentration tested mg/mL
Imipenem	1.18
Cefotaxime	0.9
Vancomycin	3.5
Dopamine	0.13
Noradrenaline	0.002
Dobutamine	0.0112
Furosemide	0.02
Fentanyl	0.01

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

1.5-5000 pg/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 1.5 pg/mL. Values above the measuring range are reported as > 5000 pg/mL (or up to 50000 pg/mL for 10-fold diluted samples).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 1.0 pg/mL

Limit of Detection = 1.5 pg/mL

Limit of Quantitation = 3.5 pg/mL

The Limit of Blank, Limit of Detection and 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 which 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

Samples with IL-6 concentrations above the measuring range can be diluted with Diluent MultiAssay. The recommended dilution is 1:10 (either automatically by the analyzers or manually). The concentration of the diluted sample must be ≥ 450 pg/mL.

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzers, the software automatically takes the dilution into account when calculating the sample concentration.

Expected values

In an external study using the Elecsys IL-6 assay on samples from 817 apparently healthy individuals a reference range up to 7 pg/mL (95th percentile) was determined.

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

Clinical performance

Measurements were performed on samples from 281 ICU patients with either a known or suspected infection. The patients were classified into categories based on the ACCP/SCCM (American College of Chest Physicians/Society of Critical Care Medicine) consensus criteria: SIRS, sepsis, severe sepsis and septic shock.[15] The IL-6 values of the patients with SIRS (n = 94) or sepsis (n = 65), severe sepsis (n = 60) or septic shock (n = 62) were as follows (3 European centers):

	IL-6 (pg/mL)					
	Median	Mean	Minimum	Maximum	N = 281	N
SIRS	62.1	150	≤ 1.5	2062	94	159
Sepsis	131	294	6.47	3122	65	
Severe sepsis	346	1827	15.2	39121	60	122
Septic shock	659	8835	8.55	171257	62	

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, samples and controls in 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 pg/mL	SD pg/mL	CV %	SD pg/mL	CV %
Human serum 1	3.47	0.141	4.1	0.180	5.2
Human serum 2	6.10	0.179	2.9	0.209	3.4
Human serum 3	21.8	0.321	1.5	0.360	1.6
Human serum 4	2592	14.5	0.6	30.8	1.2
Human serum 5	4691	45.0	1.0	62.8	1.3
PC[b)] Multimarker 1	37.2	0.378	1.0	0.650	1.7
PC Multimarker 2	241	2.33	1.0	3.66	1.5

b) PC = PreciControl

Method comparison

a) A comparison of the Elecsys IL-6 assay, REF 09015612190 (**cobas e** 801 analyzer; y), with the Elecsys IL-6 assay, REF 07027532190 (**cobas e** 801 analyzer; x), gave the following correlations (pg/mL):

Number of samples measured: 122

Passing/Bablok[16]	Linear regression
y = 1.00x + 0.457	y = 1.00x + 4.40
τ = 0.992	r = 1.00

The sample concentrations were between 1.62 and 4955 pg/mL.

b) A comparison of the Elecsys IL-6 assay, REF 09015612190 (**cobas e** 402 analyzer; y), with the Elecsys IL-6 assay, REF 09015612190 (**cobas e** 801 analyzer; x), gave the following correlations (pg/mL):

Number of samples measured: 131

Passing/Bablok[16]	Linear regression
y = 1.02x - 0.104	y = 1.02x - 0.367
τ = 0.987	r = 1.00

The sample concentrations were between 1.74 and 4853 pg/mL.

Analytical specificity

The Elecsys IL-6 assay does not show any significant cross-reactivity with the following substances, tested with IL-6 concentrations of approximately 3 pg/mL and 4000 pg/mL (max. tested concentration):

Substances	**Non-interfering concentrations (ng/mL)**
Interleukin-1α	50
Interleukin-1β	50
Interleukin-2	50
Interleukin-3	50
Interleukin-4	50
Interleukin-8	50
Interferon-γ	50
TNF-α	50

References

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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.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

The Summary of Safety & Performance Report can be found here: https://ec.europa.eu/tools/eudamed

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

CE 0123

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