Elecsys Insulin

cobas®

REF		Σ	SYSTEM
07027559190	07027559501	100	**cobas e** 402 **cobas e** 801

English

For use in the USA only

System information

Short name	ACN (application code number)
INSULIN	10059

Intended use

Immunoassay for the in vitro quantitative determination of human insulin in human serum and plasma. The determination of insulin is utilized in the diagnosis and therapy of various disorders of carbohydrate metabolism, including diabetes mellitus and hypoglycemia.

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

Summary

Insulin is a peptide hormone with a molecular weight of approximately 6000 daltons. It is secreted by the B-cells of the pancreas and passes into circulation via the portal vein and the liver. Insulin is generally released in pulses, with the parallel glucose cycle normally about 2 minutes ahead of the insulin cycle.[1]

The insulin molecule consists of two polypeptide chains, the α-chain with 21 and the β-chain with 30 amino acids. Biosynthesis of the hormone takes place in the β-cells of the islets of Langerhans in the form of single-chain preproinsulin, which is immediately cleaved to give proinsulin. Specific proteases cleave proinsulin to insulin and C-peptide which pass into the bloodstream simultaneously. About half of the insulin, but virtually none of the C-peptide, is retained in the liver. Circulating insulin has a half-life of 3-5 minutes and is preferentially degraded in the liver, whereas inactivation or excretion of proinsulin and C-peptide mainly takes place in the kidneys.

The amino acid sequence of insulin has remained surprisingly constant during evolution, with the result that prior to the development of genetically engineered human insulin it was possible to successfully use porcine or bovine insulin in the therapy of diabetes mellitus.[2]

The action of insulin is mediated by specific receptors and primarily consists of facilitation of the uptake of sugar by the cells of the liver, fatty tissue and musculature; this is the basis of its hypoglycemic action.

Serum insulin determinations are mainly performed on patients with symptoms of hypoglycemia. They are used to ascertain the glucose/insulin quotients and for clarification of questions concerning insulin secretion, e.g. in the tolbutamide test and glucagon test or in the evaluation of oral glucose tolerance tests or hunger provocation tests.

Although the adequacy of pancreatic insulin synthesis is frequently assessed via the determination of C-peptide, it is still generally necessary to determine insulin. For example, therapeutic administration of insulins of non-human origin can lead to the formation of anti-insulin antibodies. In this case, measurement of the concentration of serum insulin shows the quantity of free - and hence biologically active - hormone, whereas the determination of C-peptide provides a measure of the patient's total endogenous insulin secretion.[3,4,5]

A disorder in insulin metabolism leads to massive influencing of a number of metabolic processes. A too low concentration of free, biologically active insulin can lead to the development of diabetes mellitus. Possible causes of this include destruction of the β-cells (type I diabetes), reduced activity of the insulin or reduced pancreatic synthesis (type II), circulating antibodies to insulin, delayed release of insulin or the absence (or inadequacy) of insulin receptors.

On the other hand, autonomous, non-regulated insulin secretion is generally the cause of hypoglycemia. This condition is brought about by inhibition of gluconeogenesis, e.g. as a result of severe hepatic or renal failure, islet cell adenoma, or carcinoma. Hypoglycemia can, however, also be facilitated intentionally or unintentionally (factitious hypoglycemia).

In 3 % of persons with reduced glucose tolerance, the metabolic state deteriorates towards diabetes mellitus over a period of time. Reduced glucose tolerance during pregnancy always requires treatment. The clearly elevated risk of mortality for the fetus necessitates intensive monitoring.

The Elecsys Insulin assay employs two monoclonal antibodies which together are specific for human insulin.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: Insulin from 12 µL sample, a biotinylated monoclonal insulin-specific antibody, and a monoclonal insulin-specific antibody labeled with a ruthenium complex[a)] 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 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 INSULIN.

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

R1 Anti-insulin-Ab~biotin, 1 bottle, 10.3 mL:
Biotinylated monoclonal anti-insulin antibody (mouse) 1 mg/L; MES[b)] buffer 50 mmol/L, pH 6.0; preservative.

R2 Anti-insulin-Ab~$Ru(bpy)_3^{2+}$, 1 bottle, 9.5 mL:
Monoclonal anti-insulin antibody (mouse) labeled with ruthenium complex 1.75 mg/L; MES buffer 50 mmol/L, pH 6.0; preservative.

b) MES = 2-morpholino-ethane sulfonic acid

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.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

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 dust/fume/gas/mist/vapours/spray.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

Response:

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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: 1-800-428-2336

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.

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

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.

Criterion: Slope 0.9-1.1 + intercept within ≤ ± 0.8 µU/mL + coefficient of correlation ≥ 0.95.

Stable for 4 hours at 20-25 °C, 2 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, 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.

Sample stability claims were established by experimental data by the manufacturer or based 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.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 12017504122, Insulin CalSet, for 4 x 1.0 mL
- REF 05341787160, PreciControl Multimarker, for 6 x 2.0 mL or REF 11731416160, PreciControl Universal, for 4 x 3.0 mL
- General laboratory equipment
- **cobas e** analyzer

Additional materials for the **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 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 11298500160, 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 using the 1st IRP WHO Reference Standard 66/304 (NIBSC).

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).

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 reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Multimarker or 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 **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.

Please note: Commercial controls may contain insulin of animal origin. When assessing results, the corresponding cross-reactivity of this test must be taken into account; see under "Analytical specificity".

Calculation

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

Conversion factors: µU/mL x 6.945 = pmol/L

pmol/L x 0.144 = µU/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.

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Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1539 µmol/L or ≤ 90 mg/dL
Intralipid	≤ 1800 mg/dL
Biotin	≤ 246 nmol/L or ≤ 60 ng/mL
Rheumatoid factors	≤ 1200 IU/mL

Criterion: For concentrations of 0.4-2 µU/mL the deviation is ≤ 0.5 µU/mL. For concentrations > 2 µU/mL the deviation is ≤ 10 %.

Hemolysis interferes, as insulin-degrading peptidases are released from erythrocytes.[6]

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.

There is no high-dose hook effect at insulin concentrations up to 20000 µU/mL or 138900 pmol/L.

Pharmaceutical substances
In vitro tests were performed on 16 commonly used pharmaceuticals. Of these, only acetylcysteine at therapeutic dosage levels showed interference with the assay (insulin values depressed).

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

Special drugs

Drug	Concentration tested (mg/L)
Euglucon	10.5
Tolbutamide	3

Samples from patients treated with bovine, porcine or human insulin sometimes contain anti-insulin antibodies.[7,5] Insulin bound to these antibodies is at least partially recognized by the antibodies used in the Elecsys Insulin assay.[8]

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

0.4-1000 µU/mL or 2.78-6945 pmol/L (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.4 µU/mL (< 2.78 pmol/L). Values above the measuring range are reported as > 1000 µU/mL (> 6945 pmol/L).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.2 µU/mL (1.39 pmol/L)

Limit of Detection = 0.4 µU/mL (2.78 pmol/L)

Limit of Quantitation = 1 µU/mL (6.95 pmol/L)

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

Not necessary due to the broad measuring range.

Expected values

Studies with the Elecsys Insulin assay conducted in a clinical center in Germany with samples from 57 healthy, fasting individuals gave the following results (5th-95th percentile range):

2.6-24.9 µU/mL (17.8-173 pmol/L)

Status: Elecsys Insulin MCE, study No.: B99P027 of 29 March 2001.

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 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 µU/mL	SD µU/mL	CV %	SD µU/mL	CV %
Human serum 1	22.1	0.310	1.4	0.466	2.1
Human serum 2	3.25	0.141	4.3	0.172	5.3
Human serum 3	49.7	0.403	0.8	0.719	1.4
Human serum 4	505	5.49	1.1	8.19	1.6
Human serum 5	973	10.4	1.1	14.2	1.5
PC[c)] Multimarker 1	20.2	0.280	1.4	0.400	2.0
PC Multimarker 2	66.8	0.692	1.0	1.06	1.6

c) PC = PreciControl

cobas e 402 and **cobas e** 801 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean pmol/L	SD pmol/L	CV %	SD pmol/L	CV %
Human serum 1	153	2.15	1.4	3.24	2.1
Human serum 2	22.6	0.979	4.3	1.19	5.3
Human serum 3	345	2.80	0.8	4.99	1.4
Human serum 4	3507	38.1	1.1	56.9	1.6
Human serum 5	6757	72.2	1.1	98.6	1.5
PC Multimarker 1	140	1.94	1.4	2.78	2.0
PC Multimarker 2	464	4.81	1.0	7.36	1.6

Method comparison

A comparison of the Elecsys Insulin assay on the **cobas e** 801 analyzer (y) with the Elecsys Insulin assay on the **cobas e** 601 analyzer (x) gave the following correlations (µIU/mL):

Number of samples measured: 164

Passing/Bablok[9]	Linear regression
y = 0.988x - 0.0480	y = 0.973x + 1.09
τ = 0.993	r = 1.000

The sample concentrations were between 0.924 and 989 µIU/mL.

Analytical specificity

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

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Cross-reactant	Concentration tested	Cross-reactivity %
Bovine insulin	20000 pmol/L	9.2
Porcine insulin	10000 pmol/L	22.2
Human proinsulin	111083 pmol/L	0.36
C-peptide	33109 pmol/L	n. d.[d)]
Glucagon	288 pmol/L	n. d.
Somatostatin	60 pmol/L	n. d.
Insulin-like growth factor I	10000 pmol/L	n. d.

d) n. d. = not detectable

Results for cross-reactivity with recombinant insulin analogs in a number of insulin methods have been published for example by two groups in France and the USA.[8,10,11] The following results were published by Owen et al.[10] for the Elecsys Insulin assay:

Insulin lispro, insulin aspart, and insulin glargine were each tested in concentrations of 30, 100, 300, and 1000 mIU/L in the absence of insulin. The results obtained were below the detection limit of the Elecsys Insulin assay (< 0.4 µU/mL or < 2.78 pmol/L) at all the concentrations tested.

Moreover, these results also correlate with those published earlier by Sapin et al. for insulin lispro.[8]

References

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10 Owen WE, Roberts WL. Letter to the Editor: Cross-Reactivity of Three Recombinant Insulin Analogs with Five Commercial Insulin Immunoassays. Clin Chem 2004;50(1):257-259.

11 Sapin R. Review: Insulin Assays: Previously Known and New Analytical Features. Clin Lab 2003;49(3-4):113-121.

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

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.

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 for reconstitution
GTIN	Global Trade Item Number

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

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