

REF			SYSTEM
07027516190*	07027516500	100	cobas e 402
07027516214*			cobas e 801

* Some kits shown may not be available in all countries.

English

System information

Short name	ACN (application code number)
IGE 2	10057

Intended use

Immunoassay for the in vitro quantitative determination of immunoglobulin E in human serum and plasma.

Determination of total IgE is useful as an aid in the diagnosis of allergic diseases.

The electrochemiluminescence immunoassay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

Summary

Immunoglobulin E (IgE) plays an important role in immunological protection against parasitic infections and in allergy (type 1 hypersensitivity). Type 1 hypersensitivity is characterized by the occurrence of allergic reactions immediately following re-exposure to an allergy-initiating antigen (allergen) such as encountered in atopic disorders (e.g., allergic asthma), insect venom or latex and in some food allergies. The binding of the allergen to sensitized tissue mast cells or blood basophilic cells leads to cross-linking of the IgE on the cell membrane. This in turn causes cell degranulation and the release of inflammation mediators (e.g. histamine, serotonin, lipid mediators, proteases and cytokines), which produce the typical symptoms of type 1 hypersensitivity, an exaggerated immune response to foreign antigens, such as pollen, dust mites, and certain foods.^{1,2,3,4,5}

The IgE concentration in serum is normally very low as IgE is the least abundant antibody in serum (0.05 % of the IgG concentration). The IgE concentration is age-dependent, with the lowest values being measured at birth. Its concentration gradually increases and becomes stabilized between the age of 5-7, although the IgE values vary greatly within particular age groups.^{1,6}

Elevated IgE concentrations can be found in patients with allergic diseases such as hay fever, atopic bronchitis and dermatitis.⁴ Normal IgE values do not, however, mean that an allergic disease can be ruled out. For this reason the quantitative determination of serum IgE concentrations is useful for clinical differentiation between atopic (i.e., predisposition to excessive IgE reaction) and non-atopic (non-IgE mediated) allergic diseases only in combination with other clinical findings.^{1,6,7}

Elevated serum IgE concentrations can also occur in non-allergic diseases, e.g. congenital immunodeficiency syndromes, HIV infection, graft-versus-host disease, severe burns and parasitic diseases.⁴

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: IgE in the sample (6 µL), a biotinylated monoclonal IgE-specific antibody, and a monoclonal IgE-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)₃²⁺)

Reagents - working solutions

The **cobas e** pack is labeled as IGE 2.

- M Streptavidin-coated microparticles, 1 bottle, 6.4 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- R1 Anti-IgE-Ab-biotin, 1 bottle, 9.9 mL:
Biotinylated monoclonal anti-IgE antibody (mouse) 2.5 mg/L;
phosphate buffer 85 mmol/L, pH 6.5; preservative.
- R2 Anti-IgE-Ab-Ru(bpy)₃²⁺, 1 bottle, 9.9 mL:
Monoclonal anti-IgE antibody (mouse) labeled with ruthenium complex 5.5 mg/L; phosphate buffer 85 mmol/L, pH 6.5; 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.

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:

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.

Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

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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, K₂-EDTA and K₃-EDTA plasma.

Plasma tubes containing separating gel can be used.

Criterion: Slope 0.9-1.1 + intercept within ± 0.2 IU/mL + coefficient of correlation ≥ 0.95 .

Stable for 7 days at 20-25 °C, 7 days at 2-8 °C, 6 months at -20 °C (± 5 °C). The samples may be frozen 5 times.

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] 11930427122, IgE CalSet, 4 x 1.0 mL
- [REF] 11731416190, PreciControl Universal, for 4 x 3.0 mL
- [REF] 07299001190, Diluent Universal, 36 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 2nd WHO International Reference Preparation of Human Serum IgE (NIBSC code: 75/502).

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 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.

Calculation

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

$$\begin{aligned} \text{Conversion factors:} \quad & \text{IU/mL} \times 2.40 = \text{ng/mL} \\ & \text{ng/mL} \times 0.42 = \text{IU/mL} \end{aligned}$$

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	$\leq 633 \mu\text{mol/L}$ or $\leq 37 \text{ mg/dL}$
Hemoglobin	$\leq 0.062 \text{ mmol/L}$ or $\leq 100 \text{ mg/dL}$
Intralipid	$\leq 2200 \text{ mg/dL}$
Biotin	$\leq 409 \text{ nmol/L}$ or $\leq 100 \text{ ng/mL}$
Rheumatoid factors	$\leq 1500 \text{ IU/mL}$

Criterion: For concentrations $\leq 3 \text{ IU/mL}$ ($\leq 7.2 \text{ ng/mL}$) the deviation is $\leq 0.3 \text{ IU/mL}$ ($\leq 0.72 \text{ ng/mL}$). For concentrations $> 3 \text{ IU/mL}$ ($> 7.2 \text{ ng/mL}$) the deviation is $\leq 10 \%$.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. $> 5 \text{ mg/day}$) until at least 8 hours following the last biotin administration.

There is no high-dose hook effect at IgE concentrations up to 50000 IU/mL (120000 ng/mL).

Pharmaceutical substances

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

An interference was found for samples from patients treated with omalizumab (Xolair). Do not use samples from patients under treatment with omalizumab or similar drugs containing anti-IgE antibodies.

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.

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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

0.200-2500 IU/mL or 0.480-6000 ng/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.200 IU/mL or < 0.480 ng/mL. Values above the measuring range are reported as > 2500 IU/mL or > 6000 ng/mL (or up to 50000 IU/mL or 120000 ng/mL for 20-fold diluted samples).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.100 IU/mL (0.240 ng/mL)

Limit of Detection = 0.200 IU/mL (0.480 ng/mL)

Limit of Quantitation = 0.800 IU/mL (1.92 ng/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 $n \geq 60$ 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 IgE concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:20 (either automatically by the analyzers or manually). The concentration of the diluted sample must be > 125 IU/mL (> 300 ng/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

The IgE concentrations in healthy, non-atopic subjects are greatly dependent on age. The lowest values are found in neonates. Normal values raise in childhood and decrease again in adults.^{8,9,10} Recommended threshold values:¹⁰

Age group	IU/mL	ng/mL
Neonates	1.5	3.6
Infants in 1st year of life	15	36
Children aged 1-5 years	60	144
Children aged 6-9 years	90	216
Children aged 10-15 years	200	480
Adults	100	240

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					
Sample	Mean IU/mL	Repeatability		Intermediate precision	
		SD IU/mL	CV %	SD IU/mL	CV %
Human serum 1	0.503	0.0171	3.4	0.0201	4.0
Human serum 2	1.46	0.0306	2.1	0.0493	3.4
Human serum 3	14.8	0.242	1.6	0.343	2.3
Human serum 4	93.9	1.55	1.7	2.03	2.2
Human serum 5	1204	25.3	2.1	36.2	3.0
Human serum 6	2078	35.6	1.7	44.2	2.1
PC ^{b)} Universal 1	98.3	1.39	1.4	2.65	2.7
PC Universal 2	282	7.29	2.6	10.4	3.7

b) PC = PreciControl

cobas e 402 and cobas e 801 analyzers					
Sample	Mean ng/mL	Repeatability		Intermediate precision	
		SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	1.21	0.0410	3.4	0.0482	4.0
Human serum 2	3.50	0.0734	2.1	0.118	3.4
Human serum 3	35.5	0.581	1.6	0.823	2.3
Human serum 4	225	3.72	1.7	4.87	2.2
Human serum 5	2890	60.7	2.1	86.9	3.0
Human serum 6	4987	85.4	1.7	106	2.1
PC Universal 1	236	3.34	1.4	6.36	2.7
PC Universal 2	677	17.5	2.6	25.0	3.7

Method comparison

a) A comparison of the Elecsys IgE II assay, [REF] 04827031190 (y) with the Elecsys IgE assay (x) using clinical samples gave the following correlations (IU/mL):

Number of samples measured: 72

Passing/Bablok¹¹ Linear regression

$$y = 0.93x + 0.14$$

$$y = 0.95x - 2.35$$

$$\tau = 0.985$$

$$r = 0.998$$

The sample concentrations were between 3 and 1755 IU/mL (7.2 and 4212 ng/mL).

b) A comparison of the Elecsys IgE II assay, [REF] 07027516190 (cobas e 801 analyzer; y) with the Elecsys IgE II assay, [REF] 04827031190 (cobas e 601 analyzer; x) gave the following correlations (IU/mL):

Number of serum samples measured: 143

Passing/Bablok¹¹ Linear regression

$$y = 0.998x - 0.007$$

$$y = 0.974x + 3.90$$

$$\tau = 0.989$$

$$r = 0.999$$

The sample concentrations were between 0.342 and 2362 IU/mL (0.821 and 5669 ng/mL).

c) A comparison of the Elecsys IgE II assay, [REF] 07027516190 (cobas e 402 analyzer; y) with the Elecsys IgE II assay, [REF] 07027516190 (cobas e 801 analyzer; x) gave the following correlations (IU/mL):

Number of serum samples measured: 145

Passing/Bablok¹¹ Linear regression

$$y = 1.02x + 0.088$$

$$y = 0.999x + 3.29$$

$$\tau = 0.988$$

$$r = 1.00$$

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The sample concentrations were between 1.76 and 2476 IU/mL (4.22 and 5942 ng/mL).

Analytical specificity

The monoclonal antibodies used are highly specific for immunoglobulin E. No cross-reactivities with the immunoglobulins G, A and M were detectable.

References

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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).

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.

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):

	Contents of kit
	Analyzers/Instruments on which reagents can be used
	Reagent
	Calibrator
	Volume for reconstitution
	Global Trade Item Number

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