Elecsys Myoglobin

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REF		Σ	SYSTEM
07027583190* 07027583214*	07027583500	100	**cobas e** 402 **cobas e** 801

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

English

System information

Short name	ACN (application code number)	Application
MYO	10028	18 minutes
MYOST	10076	9 minutes (STAT = Short Turn Around Time)

Intended use

Immunoassay for the in vitro quantitative determination of myoglobin 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

Measurements of myoglobin, performed with this device, in human serum or plasma, are used as an aid-in-diagnosis of acute myocardial infarction.

Myoglobin is a small cytoplasmic protein in striated cardiac and skeletal musculature. It is involved in the transport of oxygen within the myocytes and also serves as an oxygen reservoir. Myoglobin has a low molecular weight (17.8 kDa) and is rapidly released into the bloodstream following myocardial injury.[1,2,3]

The determination of myoglobin in serum may be used in the diagnosis of acute myocardial infarction (AMI),[4,5] early reinfarction[6,7] and successful reperfusion following lysis therapy.[1,8,9,10]

Its concentration rises already after approximately 2 hours following the occurrence of symptoms, and is therefore regarded as an early marker of myocardial infarction.[11] It is rapidly excreted from the kidneys within 24 hours.[10]

During reperfusion myoglobin concentration peaks in less than 3 hours using thrombolytic measures,[12,13,14] even within 15 minutes after primary percutaneous coronary intervention.[15] Elevated myoglobin values can also occur after skeletal muscle damage and in cases of greatly restricted renal function.[1,2] According to the 4th Universal Definition of Myocardial Infarction, cardiac troponins are the preferred biomarkers for the evaluation of myocardial injury, since other biomarkers are less specific and less sensitive. High-sensitivity cardiac troponin assays are recommended for routine clinical use.[16] In the case that high-sensitivity cardiac troponin assays are not available, a combination of other cardiac biomarkers, including myoglobin, may increase sensitivity for the diagnosis of AMI compared to single biomarkers.[17]

The Elecsys Myoglobin assay is based on the sandwich principle using two different monoclonal antibodies directed against human myoglobin.

Test principle

Sandwich principle.

Total duration of assay: 18 minutes.

- 1st incubation: Antigen in the sample (9 µL), a biotinylated monoclonal myoglobin-specific antibody, and a monoclonal myoglobin-specific antibody labeled with a ruthenium complex[a)] react to 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.

Total duration of assay: 9 minutes.

- During a 9 minute incubation, antigen in the sample (9 µL), a biotinylated monoclonal myoglobin-specific antibody, a monoclonal myoglobin-specific antibody labeled with a ruthenium complex and streptavidin-coated microparticles react to form a sandwich complex, which is bound to the solid phase.

For both assay applications:

- 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 MYO.

M Streptavidin-coated microparticles, 1 bottle, 6.1 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-myoglobin-Ab~biotin, 1 bottle, 9.9 mL:
Biotinylated monoclonal anti-myoglobin antibody (mouse) 1.75 mg/L; phosphate buffer 85 mmol/L, pH 6.5; sodium azide < 0.1 %; preservative.

R2 Anti-myoglobin-Ab~$Ru(bpy)_3^{2+}$, 1 bottle, 9.9 mL:
Monoclonal anti-myoglobin antibody (mouse) labeled with ruthenium complex 1.75 mg/L; phosphate buffer 85 mmol/L, pH 6.5; sodium azide < 0.1 %; 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 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.

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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 Elecsys Myoglobin assay can be used for both the 9-minute application and the 18-minute application.

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.

Li-heparin and K_2-EDTA plasma tubes containing separating gel can be used.

Criterion: Slope 0.9-1.1 + intercept within ≤ ± 10 ng/mL + coefficient of correlation ≥ 0.95.

Stable for 8 days at 20-25 °C, 14 days at 2-8 °C, 12 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 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 07394675190, CalSet Myoglobin, 4 x 1.0 mL
- REF 04917049190, PreciControl Cardiac II, for 4 x 2.0 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 an inhouse reference preparation.

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

Use Elecsys PreciControl Cardiac II or other suitable controls for routine quality control procedures.

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 ng/mL or µg/L.

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	≤ 1112 µmol/L or ≤ 65 mg/dL
Hemoglobin	≤ 0.869 mmol/L or ≤ 1400 mg/dL
Intralipid	≤ 2200 mg/dL
Biotin	≤ 205 nmol/L or ≤ 50 ng/mL
Rheumatoid factors	≤ 1500 IU/mL

Criterion: For concentrations of 21-50 ng/mL the deviation is ≤ 5 ng/mL. For concentrations > 50 ng/mL the deviation is ≤ 10 %.

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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 myoglobin concentrations up to 30000 ng/mL.

Above this concentration, the hook effect can be observed. In case of an unexpected low result, the sample should be diluted 1:10 (refer to section "dilution") and retested.

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

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

Special cardiac drugs

Drug	Concentration tested mg/L
Carvedilol	37.5
Clopidogrel	75.0
Digoxin	0.25
Epinephrine (Adrenaline)	0.50
Insulin	1.60
Lidocaine	80.0
Lisinopril	10.0
Methylprednisolone	7.50
Metoprolol	150
Nifedipine	30.0
Phenprocoumon	3.00
Propafenone	300
Reteplase	33.3
Simvastatin	30.0
Spironolactone	75.0
Tolbutamide	1500
Torasemide	15.0
Verapamil	240

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

21-3000 ng/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 21 ng/mL. Values above the measuring range are reported as > 3000 ng/mL (or up to 30000 ng/mL for 10-fold diluted samples).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 18 ng/mL

Limit of Detection = 21 ng/mL

Limit of Quantitation = 25 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 ≥ 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 myoglobin concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:10 (either automatically by the analyzer or manually). The concentration of the diluted sample must be > 50 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

In studies with the Elecsys Myoglobin assay on 2155 healthy test subjects the following data were obtained:

	Number	2.5-97.5th percentile
Men	1027	28-72 ng/mL
Women	1128	25-58 ng/mL

Data (status 1999) combined from: Multicenter Evaluation of the Elecsys Myoglobin STAT assay and International Elecsys 1010 Study, Cardiac Markers.

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, 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 (18-minute application)					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	25.8	0.686	2.7	0.700	2.7
Human serum 2	47.3	0.860	1.8	0.935	2.0
Human serum 3	64.5	0.949	1.5	1.08	1.7
Human serum 4	1428	21.4	1.5	23.5	1.6
Human serum 5	2495	43.5	1.7	58.9	2.4
PC[b)] Cardiac II 1	73.0	0.769	1.1	1.05	1.4
PC Cardiac II 2	986	12.0	1.2	16.0	1.6

b) PC = PreciControl

cobas e 402 and **cobas e** 801 analyzers (9-minute application)					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	24.6	0.764	3.1	0.764	3.1
Human serum 2	46.8	0.646	1.4	0.798	1.7
Human serum 3	63.9	1.19	1.9	1.32	2.1
Human serum 4	1421	26.9	1.9	29.6	2.1
Human serum 5	2527	50.6	2.0	51.6	2.0

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cobas e 402 and **cobas e** 801 analyzers (9-minute application)					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
PC Cardiac II 1	73.5	0.947	1.3	1.18	1.6
PC Cardiac II 2	983	12.4	1.3	13.4	1.4

Method comparison

a) A comparison of the Elecsys Myoglobin assay, REF 07027583190 (9-minute application; y) with the Elecsys Myoglobin assay, REF 07027583190 (18-minute application; x) on the **cobas e** 801 analyzer gave the following correlations (ng/mL):

Number of samples measured: 143

Passing/Bablok[18]	Linear regression
y = 0.979x - 0.201	y =1.01x - 13.0
τ = 0.986	r = 0.999

The sample concentrations were between 22.9 and 2826 ng/mL.

b) A comparison of the Elecsys Myoglobin assay, REF 07027583190 (9-minute application, **cobas e** 801 analyzer; y) with the Elecsys Myoglobin STAT assay, REF 11820788122 (**cobas e** 601 analyzer; x) gave the following correlations (ng/mL):

Number of samples measured: 140

Passing/Bablok[18]	Linear regression
y = 0.954x + 2.05	y = 0.951x + 3.36
τ = 0.981	r = 0.999

The sample concentrations were between 21.3 and 2898 ng/mL.

c) A comparison of the Elecsys Myoglobin assay, REF 07027583190 (**cobas e** 402 analyzer; y) with the Elecsys Myoglobin assay, REF 07027583190 (**cobas e** 801 analyzer; x) gave the following correlations (ng/mL):

Number of serum samples measured: 136

Passing/Bablok[18]	Linear regression
y = 1.04x + 1.37	y = 1.04x + 4.26
τ = 0.982	r = 1.00

The sample concentrations were between 22.2 and 2941 ng/mL.

References

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17 Fan J, Ma J, Xia N, et al., Clinical Value of Combined Detection of CK-MB, MYO, cTnI and Plasma NT-proBNP in Diagnosis of Acute Myocardial Infarction. Clin Lab 2017;63(3):427-433.

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For further information, please refer to the appropriate user guide or 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.

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

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

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