CKMB

Creatine Kinase-MB

cobas®

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08057486190	Creatine Kinase-MB (150 tests)	System-ID 2043 001	**cobas c** 303, **cobas c** 503

Materials required (but not provided):

11447394216	Calibrator f.a.s. CK-MB (3 x 1 mL)	Code 20402	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

English

System information

CKMB2: ACN 20430

Intended use

In vitro test for the quantitative determination of the catalytic activity of creatine kinase MB subunit (CK-MB) in human serum and plasma on **cobas** c systems.

Summary

Creatine kinase (CK) appears as three isoenzymes which are dimers composed of two types of monomer subunits. The isoenzymes comprise all three combinations of monomers, M (for skeletal muscle derived) and B (for brain derived), as represented by the notations MM, MB, and BB.[1]

Many organs contain CK, but the distribution of isoenzymes is different in each one. Skeletal muscle is very rich in the MM isoenzyme, while brain, stomach, intestine, bladder, and lung contain primarily the BB isoenzyme. The MB isoenzyme has been found in appreciable amounts (15 to 20 percent) only in myocardial tissue. Therefore, total serum CK activity is elevated in a number of diseases. This lack of specificity limits its diagnostic value. However, the striking difference in the CK isoenzyme patterns from different organs has made CK one of the most useful enzymes for diagnostic purposes in acute myocardial infarction. CK-MB appears in serum reflecting its unique presence in myocardial tissue. It is in supporting the diagnosis of suspected myocardial infarction that serial determinations of CK isoenzymes find their most frequent application in the clinical laboratory.[1,2]

After immunoinhibition with antibodies to the CK-M subunit,[3] the CK-B activity is determined with a standardized method for the determination of CK with activation by NAC as recommended by the German Society for Clinical Chemistry (DGKC)[4] and the International Federation of Clinical Chemistry (IFCC)[5,6] in 1977 and 2002 respectively. This assay meets the recommendations of the IFCC and DGKC, but was optimized for performance and stability.

Test principle

Immunological UV assay

- Sample and addition of R1 (buffer/enzymes/coenzyme)
- Addition of R2 (buffer/substrate/antibody) and start of reaction.

Human CK-MB is composed of two subunits, CK-M and CK-B which both have an active site. With the aid of specific antibodies to CK-M, the catalytic activity of CK-M subunits in the sample is inhibited to 99.6 % without affecting the CK-B subunits. The remaining CK-B activity, corresponding to half the CK-MB activity, is determined by the total CK method. As the CK-BB isoenzyme only rarely appears in serum and the catalytic activity of the CK-M and CK-B subunits hardly differ, the catalytic activity of the CK-MB isoenzyme can be calculated from the measured CK-B activity by multiplying the result by 2.

Reagents - working solutions

R1 Imidazole buffer: 123 mmol/L, pH 6.5 (37 °C); EDTA: 2.46 mmol/L; Mg^{2+}: 12.3 mmol/L; ADP: 2.46 mmol/L; AMP: 6.14 mmol/L; diadenosine pentaphosphate: 19 µmol/L; NADP (yeast): 2.46 mmol/L; N-acetylcysteine: 24.6 mmol/L; HK (yeast): ≥ 36.7 µkat/L; G6P-DH (E. coli): ≥ 23.4 µkat/L; preservative; stabilizers; additives.

R2 CAPSO* buffer: 20 mmol/L, pH 8.8 (37 °C); glucose: 120 mmol/L; EDTA: 2.46 mmol/L; creatine phosphate: 184 mmol/L; 4 monoclonal anti-CK-M antibodies (mouse), inhibiting capacity: > 99.6 % up to 66.8 µkat/L (4000 U/L) (37 °C) CK-M subunit; preservative; stabilizers; additive.

*CAPSO: 3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid

R1 is in position B and R2 is in position C.

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:

Danger

H360D	May damage the unborn child.
Prevention:	
P201	Obtain special instructions before use.
P202	Do not handle until all safety precautions have been read and understood.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.
Response:	
P308 + P313	IF exposed or concerned: Get medical advice/attention.
Storage:	
P405	Store locked up.
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

Reagent handling

Ready for use

CKMB

Creatine Kinase-MB

cobas®

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	8 weeks

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Serum: Nonhemolyzed serum is the specimen of choice and also recommended by IFCC.
Plasma: Li-heparin, K_2-, K_3-EDTA plasma.

Li-heparin in the usual concentration does not interfere with the test, but IFCC warns against its use.[5]

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.

See the limitations and interferences section for details about possible sample interferences.

Stability in serum:[7]	8 hours at 20-24 °C
	8 days at 2-8 °C
	4 weeks at –20 °C
Stability in heparin plasma:[7]	8 hours at 20-24 °C
	5 days at 2-8 °C
	8 days at –20 °C
Stability in EDTA plasma:[8]	2 days at 20-25 °C
	7 days at 4-8 °C
	1 year at –20 °C

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section

General laboratory equipment

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.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

Test definition

Reporting time	10 min		
Wavelength (sub/main)	546/340 nm		
Reagent pipetting		Diluent (H_2O)	
R1	79 µL	–	
R2	16 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	3.9 µL	–	–
Decreased	11.7 µL	10 µL	80 µL
Increased	3.9 µL	–	–

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s. CK-MB
Calibration mode	Linear
Calibration frequency	Automatic full calibration - after reagent lot change
	Full calibration - as required following quality control procedures

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

Traceability: This method has been standardized against the IFCC Method for Creatine Kinase[6] with addition of antibodies.

Quality control

For quality control, use control materials as listed in the “Order information” section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory’s individual requirements. It is recommended to perform quality control always after lot calibration and subsequently at least every 8 weeks. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

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

Calculation

cobas c systems automatically calculate the analyte activity of each sample in the unit U/L (µkat/L).

Conversion factor: U/L × 0.0167 = µkat/L

Limitations - interference

The total CK activity of the specimen should be determined prior to performing the CK-MB assay. The amount of anti-human CK-M subunit antibody in the CK-MB reagent is sufficient for the complete inhibition of up to 4000 U/L CK-M activity. If the total CK activity exceeds 4000 U/L, the specimen requires dilution because complete inhibition of the CK-M subunit is no longer assured. In patients with a disposition to macro-CK formation, implausibly high CK-MB values may be measured in relation to the total CK, since the macroforms mainly consist of CK-B subunits. As these patients have generally not suffered a myocardial infarction, additional diagnostic measures are necessary.[9]

Criterion: Recovery within ± 10 % of initial value at a CK-MB activity of ≥ 25 U/L.

Icterus:[10] No significant interference up to an I index of 60 for conjugated and 20 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL and approximate unconjugated bilirubin concentration: 342 µmol/L or 20 mg/dL).

Hemolysis:[10] No significant interference up to an H index of 20 (approximate hemoglobin concentration: 12.4 µmol/L or 20 mg/dL).

Lipemia (Intralipid):[10] No significant interference up to an L index of 500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Adenylate kinase: Adenylate kinase (AK) may cause positive interference. Sources of AK in the blood are erythrocytes, muscle, and liver. In order to reduce AK interference to a minimum, AMP and Ap_5A are included in the reagent. The AMP/Ap_5A mixture causes 97 % inhibition of the AK from erythrocytes and muscle, and 95 % inhibition of the AK from liver.[4] The slight residual AK activity does not influence the assay of total CK, but may affect the low CK-MB activities.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[11,12] Exceptions: Cyanokit (hydroxocobalamin) and cefoxitin at therapeutic concentrations interfere with the test.

In very rare cases, gammopathy, in particular type IgM (Waldenström’s macroglobulinemia), may cause unreliable results.[13]

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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.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

Limits and ranges

Measuring range

3-2000 U/L (0.05-33.4 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:3 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 3.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 3 U/L (0.05 µkat/L)

Limit of Detection = 3 U/L (0.05 µkat/L)

Limit of Quantitation = 5 U/L (0.08 µkat/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 activity 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 activity samples.

The Limit of Detection corresponds to the lowest analyte activity which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte activity that can be reproducibly measured with a total error of 20 %. It has been determined using low activity CK-MB samples.

Expected values

Reference intervals strongly depend on the patient group regarded and the specific clinical situation.

U/L

For healthy people: Reference range (37 °C) according to Klein et al.[14] and consensus values:[15]

< 25 U/L

For myocardial infarction diagnosis using the combination CK and CK-MB (activity), and representing a CK consensus value based on long-term experience:[15,16]

1. CK_{men} > 190 U/L
   CK_{women} > 167 U/L
2. CK-MB > 24 U/L
3. The CK-MB activity accounts for 6-25 % of the total CK activity.

µkat/L

For healthy people: Reference range (37 °C) according to Klein et al.[14] and consensus values:[15]*

< 0.418 µkat/L

*calculated by unit conversion factor

For myocardial infarction diagnosis using the combination CK and CK-MB (activity), and representing a CK consensus value based on long-term experience:[15,16]

1. CK_{men} > 3.17 µkat/L
   CK_{women} > 2.79 µkat/L
2. CK-MB > 0.40 µkat/L
3. The CK-MB activity accounts for 6-25 % of the total CK activity.

When myocardial infarction is suspected the diagnostic strategy proposals in the consensus document of European and American cardiologists should in general be followed.[17]

If despite the suspicion of myocardial infarction the values found remain below the stated limits, a fresh infarction may be involved. In such cases the determinations should be repeated after 4 hours.

Maximum diagnostic efficiency of the CK-MB determination will be obtained when a sequential sampling protocol is used and consideration is given to the time pattern of activity over a 6 to 48 hour period. When only CK-MB activity is used, the diagnostic efficiency will be lower and will vary with the sampling time.[1,9]

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. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

Repeatability	*Mean* *U/L*	*SD* *U/L*	*CV* *%*
PCCC1[a)]	42.9	0.380	0.9
PCCC2[b)]	96.9	0.365	0.4
Human serum 1	16.0	0.358	2.2
Human serum 2	22.6	0.365	1.6
Human serum 3	190	0.779	0.4
Human serum 4	997	2.61	0.3
Human serum 5	1782	4.80	0.3

Intermediate precision	*Mean* *U/L*	*SD* *U/L*	*CV* *%*
PCCC1[a)]	42.9	0.557	1.3
PCCC2[b)]	96.6	0.712	0.7
Human serum 1	15.5	0.507	3.3
Human serum 2	22.3	0.560	2.5
Human serum 3	190	3.24	1.7
Human serum 4	997	11.1	1.1
Human serum 5	1784	28.1	1.6

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s).

Method comparison

CK-MB values for human serum and plasma samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 69

Passing/Bablok[18]	Linear regression
y = 1.014x – 1.73 U/L	y = 1.013x – 1.24 U/L

τ = 0.964 r = 1.000

The sample activities were between 4.90 and 1876 U/L.

CK-MB values for human serum and plasma samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 69

Passing/Bablok[18]	Linear regression
y = 1.015x + 0.202 U/L	y = 1.023x + 0.108 U/L
τ = 0.932	r = 1.000

The sample activities were between 3.50 and 1970 U/L.

References

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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
→	Volume for reconstitution
GTIN	Global Trade Item Number

CE 0123

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