CKMBL

cobas®

Creatine Kinase-MB

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04525299 190	Creatine Kinase-MB 100 tests	System-ID 07 5924 4	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
11447394 216	Calibrator f.a.s. CK-MB (3 x 1 mL)	Code 402	
11447378 122	Precinorm CK-MB (4 x 3 mL)	Code 320	
04358210 190	Precipath CK-MB (4 x 3 mL, not available in the USA)	Code 356	
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05947626 160	PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	Code 391	
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	
05947774 160	PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	Code 392	
04489357 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3	

English

System information

For **cobas c** 311/501 analyzers:

CKMBL: ACN 060

For **cobas c** 502 analyzer:

CKMB: ACN 8060

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 Roche/Hitachi **cobas c** systems.

Summary[1,2,3,4,5,6,7]

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.

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 (15-20 %) 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.

After immunoinhibition with antibodies to the CK-M subunit, the CK-B activity is determined with a standardized method for the determination of CK using the “reverse reaction” and activation by NAC as recommended by the German Society for Clinical Chemistry (DGKC) and the International Federation of Clinical Chemistry (IFCC) 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

The CK-M subunits are inhibited by specific antibodies. Since CK-BB occurs rarely in serum it is assumed that the CK-B activity is derived from CK-MB present in the specimen. The activity of the CK-B subunits is determined and multiplied by 2 to provide an estimate of the CK-MB activity. The CK is activated by N-acetylcysteine (NAC). In a primary reaction, the activated CK catalyzes the dephosphorylation of creatine phosphate to form creatine and ATP. In a coupled reaction catalyzed by hexokinase (HK), glucose is phosphorylated by ATP to form D-glucose-6-phosphate (G6P). Finally, glucose-6-phosphate dehydrogenase (G6PDH) catalyzes the oxidation of G6P by $NADP^+$ to form 6-phosphogluconate and NADPH.

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow{\text{CK}} \text{creatine} + \text{ATP}$$

$$\text{ATP} + \text{D-glucose} \xrightarrow{\text{HK}} \text{ADP} + \text{G6P}$$

$$\text{G6P} + \text{NADP}^+ \xrightarrow{\text{G6PDH}} \text{D-6-phosphogluconate} + \text{NADPH} + \text{H}^+$$

The rate of the NADPH formation is directly proportional to the catalytic CK-MB activity. It is determined by measuring the increase in absorbance photometrically.

Reagents - working solutions

R1 Imidazole: 58.0 mmol/L, pH 6.0; N-acetylcysteine: 40.0 mmol/L; EDTA: 3.0 mmol/L; AMP: 10.0 mmol/L; diadenosine pentaphosphate: 24.0 µmol/L; $NADP^+$: 9.5 mmol/L; Mg^{2+}: 20.0 mmol/L; D-glucose: 40.0 mmol/L; stabilizer

R2 EDTA: 3.0 mmol/L, pH 9.1; HK (yeast): ≥ 600 µkat/L; G6PDH (microbial): ≥ 600 µkat/L; ADP: 12.0 mmol/L; creatine phosphate: 180 mmol/L; N-methyldiethanolamine: 69.0 mmol/L; monoclonal murine antibodies inhibiting human CK-M (inhibiting capacity ≥ 2000 U/L of CK-MM); preservative; stabilizer; detergent

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

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

For USA: For prescription use only.

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.

P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.

Response:

P308 + P313 IF exposed or concerned: Get medical advice/attention.

Disposal:

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P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling primarily follows EU GHS guidance.
Contact phone: all countries: +49-621-7590, USA: 1-800-428-2336

Reagent handling
Ready for use

Storage and stability

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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

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 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 (free from hemolysis). Nonhemolyzed serum is the specimen of choice and also recommended by IFCC.
Plasma (free from hemolysis): Li-heparin plasma
Li-heparin in the usual concentration does not interfere with the test, but IFCC warns against its use.[8]

Do not use plasma prepared with other anticoagulants.

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.

Stability in serum:[9]	8 hours at 20-24 °C
	8 days at 2-8 °C
	4 weeks at -20 °C
Stability in heparin plasma:[9]	8 hours at 20-24 °C
	5 days at 2-8 °C
	8 days 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

cobas c 311 **test definition**

Assay type	Rate A
Reaction time / Assay points	10/37-56
Wavelength (sub/main)	546/340 nm
Reaction direction	Increase
Units	U/L (µkat/L)
Reagent pipetting	Diluent (H_2O)
R1	61 µL 24 µL
R2	20 µL –

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	16.5 µL	–	–
Decreased	8.3 µL	–	–
Increased	16.5 µL	–	–

cobas c 501 **test definition**

Assay type	Rate A
Reaction time / Assay points	10/52-70
Wavelength (sub/main)	546/340 nm
Reaction direction	Increase
Units	U/L (µkat/L)
Reagent pipetting	Diluent (H_2O)
R1	61 µL 24 µL
R2	20 µL –

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	16.5 µL	–	–
Decreased	8.3 µL	–	–
Increased	16.5 µL	–	–

cobas c 502 **test definition**

Assay type	Rate A
Reaction time / Assay points	10/52-70
Wavelength (sub/main)	546/340 nm
Reaction direction	Increase
Units	U/L (µkat/L)
Reagent pipetting	Diluent (H_2O)
R1	61 µL 24 µL
R2	20 µL –

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	16.5 µL	–	–
Decreased	8.3 µL	–	–
Increased	33.0 µL	–	–

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s. CK-MB
Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • as required following quality control procedures

Traceability: This method has been standardized against the original IFCC formulation[3] with addition of antibodies using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ε.

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. 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

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

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

Limitations - interference[10]

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 2000 U/L CK-MM activity. If the total CK activity exceeds 2000 U/L, the specimen requires dilution because complete inhibition of the CK-M subunit is no longer assured.

The CK-MB method measures not only CK-MB but also CK-BB, mitochondrial-CK or CK-BB-IgG present in patient sera. These latter sources of CK-B activity can be distinguished by persistent elevation of CK-MB over an extended time period. Electrophoresis may be used to confirm atypical CK isoenzymes.[11]

Criterion: Recovery within ± 10 % of initial value at a creatine kinase-MB activity of 25 U/L (0.42 µkat/L).

Icterus: No significant interference up to an I index of 40 for conjugated and 20 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 684 µmol/L or 40 mg/dL and approximate unconjugated bilirubin concentration: 342 µmol/L or 20 mg/dL).

Hemolysis: No significant interference up to an H index of 10 (approximate hemoglobin concentration: 6 µmol/L or 10 mg/dL).

Lipemia (Intralipid): No significant interference up to an L index of 100. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration. Lipemic specimens with an L index > 100 may cause high absorbance flagging. Choose diluted sample treatment for automatic rerun.

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.[12]

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.[6] 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.[13,14] Exceptions: Physiological plasma concentrations of Sulfasalazine or Sulfapyridine may lead to false results.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[15]

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 Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD-SMS-SmpCln1+2-SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is not required.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

3-500 U/L (0.05-8.35 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:1.99 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 1.99.

Lower limits of measurement

Lower detection limit of the test

3 U/L (0.05 µkat/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Expected values

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

For healthy people: Reference range (37 °C) according to Klein et al.[16] and consensus values:[17]

< 25 U/L (< 0.421 µkat/L)

For myocardial infarction diagnosis using the combination CK and CK-MB (activity), and representing a CK consensus value based on long-term experience:[17,18]

1. CK_{men} > 190 U/L (3.12 µkat/L)
   CK_{women} > 167 U/L (2.87 µkat/L)
2. CK-MB > 24 U/L (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.[19]

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.[2,11]

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 human samples and controls in an internal protocol with repeatability (n = 21) and intermediate precision (3 aliquots per run, 1 run per day, 21 days). The following results were obtained:

Repeatability	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm CK-MB	179 (2.99)	1 (0.02)	0.3
Human serum 1	15.0 (0.251)	0.3 (0.005)	1.7
Human serum 2	51.5 (0.860)	0.9 (0.015)	1.7
Human serum 3	141 (2.35)	1 (0.02)	0.9

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Intermediate precision	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm CK-MB	171 (2.86)	3 (0.05)	2.0
Human serum 4	12.3 (0.205)	1.0 (0.017)	8.1
Human serum 5	244 (4.07)	11 (0.18)	4.7

Method comparison

Creatine kinase-MB values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 228

Passing/Bablok[20]	Linear regression
y = 1.005x + 4.098 U/L	y = 1.046x + 2.619 U/L
τ = 0.870	r = 0.989

The sample activities were between 6.30 and 201 U/L (0.105 and 3.34 µkat/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.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

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Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

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

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Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
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