

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 COBAS INTEGRA 400 plus COBAS INTEGRA 800
11447394 216	C.f.a.s. CK-MB (3 × 1 mL)	System-ID 07 7996 2
11447378 122	Precinorm CK-MB (4 × 3 mL)	System-ID 07 9111 3
04358210 190	Precipath CK-MB* (4 × 3 mL)	System-ID 07 6828 6
05117003 190	PreciControl ClinChem Multi 1 (20 × 5 mL)	System-ID 07 7469 3
05947626 190	PreciControl ClinChem Multi 1 (4 × 5 mL)	System-ID 07 7469 3
05947626 160	PreciControl ClinChem Multi 1 (4 × 5 mL, for USA)	System-ID 07 7469 3
05117216 190	PreciControl ClinChem Multi 2 (20 × 5 mL)	System-ID 07 7470 7
05947774 190	PreciControl ClinChem Multi 2 (4 × 5 mL)	System-ID 07 7470 7
05947774 160	PreciControl ClinChem Multi 2 (4 × 5 mL, for USA)	System-ID 07 7470 7

*Not for use in the US

English

System information

Test CKMBL, test ID 0-324

Intended use

In vitro test for the quantitative determination of the catalytic activity of CK-MB (EC 2.7.3.2; adenosine triphosphate: creatine N-phosphotransferase) in human serum and plasma on COBAS INTEGRA systems.

Summary^{1,2}

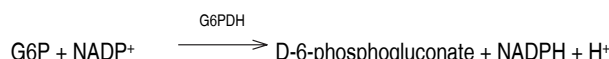
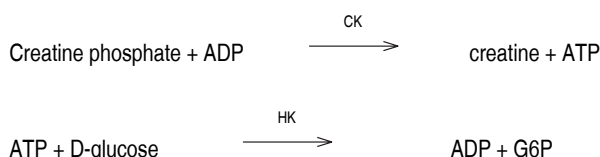
Creatine kinase (CK) appears as three isoenzymes which are dimers composed of two types of monomer subunits. The isoenzymes comprise all three combinations of monomers, M (for skeletal muscle derived) and B (for brain derived), as represented by the notations MM, MB, and BB.

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.

Test principle

After immunoinhibition with antibodies to the CK-M subunit, the CK-B activity is determined with a method according to the recommendations of the International Federation of Clinical Chemistry (IFCC), the Société Française de Biologie Clinique (SFBC), the Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology (SCE), and the Deutsche Gesellschaft für Klinische Chemie (DGKC).^{3,4,5,6,7}

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.



The rate of the NADPH formation is directly proportional to the catalytic CK-MB activity. It is determined by measuring the increase in absorbance at 340 nm.

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; Mg²⁺: 20.0 mmol/L; D-glucose: 40.0 mmol/L; stabilizer

SR 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 SR is in position C.

Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

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:

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

Shelf life at 2-8 °C See expiration date on
cobas c pack label

COBAS INTEGRA 400 plus system

On-board in use at 10-15 °C 8 weeks

COBAS INTEGRA 800 system

On-board in use at 8 °C 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 (free from hemolysis)

Nonhemolyzed serum is the specimen of choice and also recommended by IFCC.

Plasma (free from hemolysis): Li-heparin plasma

Li-heparin does not interfere with the test. Plasma prepared with this anticoagulant in the usual concentration is also acceptable. However, 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 h at 20-24 °C
8 days at 2-8 °C
4 weeks at -20 °C

Stability in Li-heparin plasma:⁹ 8 h at 20-24 °C
5 days at 2-8 °C
8 days at -20 °C

Materials provided

See "Reagents – working solutions" section for reagents.

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.

Application for serum and plasma**COBAS INTEGRA 400 plus test definition**

Measuring mode	Absorbance
Abs. calculation mode	Kinetic
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A/B	340/409 nm
Calc. first/last	53/65
Unit	U/L

Pipetting parameters

		Diluent (H ₂ O)
R1	61 µL	9 µL
Sample	16.5 µL	10 µL
SR	20 µL	5 µL

Total volume 121.5 µL

COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Kinetic
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A/B	340/409 nm
Calc. first/last	78/98
Unit	U/L

Pipetting parameters

		Diluent (H ₂ O)
R1	61 µL	9 µL
Sample	16.5 µL	10 µL
SR	20 µL	5 µL
Total volume	121.5 µL	

Calibration

Calibrator	C.f.a.s. CK-MB Use deionized water as zero calibrator.
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	Each lot and as required following quality control procedures

Traceability: This method has been standardized manually against the original IFCC formulation with addition of antibodies.

Quality control

Reference range	Precinorm CK-MB or PreciControl ClinChem Multi 1
Pathological range	Precipath CK-MB* or PreciControl ClinChem Multi 2
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

*Not for use in the US

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

COBAS INTEGRA analyzers automatically calculate the analyte activity of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

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 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. Choose diluted sample treatment for automatic rerun (postdilution factor 10). If the total CK activity exceeds 20000 U/L dilute the

specimen with 0.9 % saline solution such that the total activity is less than 2000 U/L. Multiply the results of the diluted specimen by the appropriate dilution factor. 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.¹⁰

Criterion: Recovery within ± 10 % of initial value.

Serum/plasma

Icterus:¹¹ No significant interference up to an I index of 20 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 340 $\mu\text{mol/L}$ or 20 mg/dL).

Hemolysis:¹¹ No significant interference up to an H index of 10 (approximate hemoglobin concentration: 6 $\mu\text{mol/L}$ or 10 mg/dL).

Lipemia:¹¹ Intralipid levels > 500 mg/dL may cause high absorbance flagging. Choose diluted sample treatment for automatic rerun.

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.^{12,13} Exceptions: Methyldopa, cefoxitin, and calcium dobesilate cause artificially low CK-MB activities. Physiological plasma concentrations of Sulfasalazine may lead to false results. Temozolomide at therapeutic concentrations may lead to erroneous results.

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.¹⁴

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 COBAS INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.

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 $\mu\text{kat/L}$)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 10.

Lower limits of measurement

Lower detection limit of the test:

3 U/L (0.05 $\mu\text{kat/L}$)

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 a zero sample (zero sample + 3 SD, repeatability, $n = 30$).

Expected values

Reference range (37 °C) according to Klein et al.¹⁶ and consensus values:¹⁷ < 25 U/L (< 0.421 $\mu\text{kat/L}$)

Myocardial infarction: There is high probability of myocardial damage when the following three conditions are fulfilled:¹⁸

1. CK_{men} > 190 U/L (> 3.12 $\mu\text{kat/L}$)*
 CK_{women} > 167 U/L (> 2.87 $\mu\text{kat/L}$)*

2. CK-MB > 24 U/L (> 0.40 $\mu\text{kat/L}$)*

3. The CK-MB activity accounts for 6-25 % of the total CK activity.

*Calculated with a temperature conversion factor of 2.38 (25 \rightarrow 37 °C)¹⁹

When myocardial infarction is suspected the diagnostic strategy proposals in the consensus document of European and American cardiologists should in general be followed.²⁰

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 6-48 hour period. When only CK-MB activity is used, the diagnostic efficiency will be lower and will vary with the sampling time.^{2,10}

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 COBAS INTEGRA 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 and intermediate precision (3 aliquots per run, 2 runs per day, 11 days). The following results were obtained:

	Level 1	Level 2
Mean	20 U/L (0.33 $\mu\text{kat/L}$)	117 U/L (1.95 $\mu\text{kat/L}$)
CV repeatability	1.5 %	1.9 %
CV intermediate precision	2.8 %	2.4 %

Method comparison

CK-MB values for human serum and plasma samples obtained on a COBAS INTEGRA 700 analyzer using the COBAS INTEGRA CKMBL reagent (y) were compared with those determined using the previous COBAS INTEGRA reagent (CKMB) on the same analyzer (x) and with those determined using a commercially available reagent on an alternative manufacturer's clinical chemistry system (x). Samples were measured in duplicate. Sample size (n) represents all replicates.

	COBAS INTEGRA 700 analyzer	Alternative system
Sample size (n)	140	105
Corr. coefficient (r)	0.998	0.992
Lin. regression	$y = 0.99x - 1 \text{ U/L}$	$y = 0.83x + 4 \text{ U/L}$
Passing/Bablok ²¹	$y = 0.99x - 1 \text{ U/L}$	$y = 0.84x + 3 \text{ U/L}$

The sample activities were between 5 and 214 U/L (0.08 and 3.57 $\mu\text{kat/L}$).

References

- 1 Lott JA, Stang JM. Serum enzymes and isoenzymes in the diagnosis and differential diagnosis of myocardial ischemia and necrosis. Clin Chem 1980;26:1241-1250.
- 2 Moss DW, Henderson AR, Kachmar JF. Enzymes. In: Tietz NW, ed. Fundamentals of Clinical Chemistry, 3rd ed. Philadelphia, PA: WB Saunders 1987;346-421.
- 3 Schumann G, Bonora R, Ceriotti F, et al. IFCC Primary Reference Procedures for the Measurement of Catalytic Activity Concentrations of Enzymes at 37 °C – Part 2. Reference Procedure for the Measurement of Catalytic Concentrations of Creatine Kinase. Clin Chem Lab Med 2002;40(6):635-642.
- 4 Mathieu M, Breaudiere JP, Galteau MM, et al. Recommendations for measuring the catalytic concentration of creatine kinase in human serum at 30°C. Ann Biol Clin 1982;40:138-149.
- 5 Hørdér M, Magid E, Pitkänen E, et al. Recommended method for the determination of creatine kinase in blood modified by the inclusion of EDTA. Scand J Clin Lab Invest 1979;39:1-5.

- 6 Bergmeyer HU, Breuer H, Büttner H, et al. Empfehlungen der Deutschen Gesellschaft für Klinische Chemie. Standard-Methode zur Bestimmung der Aktivität der Creatin-Kinase. J Clin Chem Clin Biochem 1977;15:249-254.
- 7 Würzburg U, Hennrich N, Lang H, et al. Determination of creatine kinase-MB in serum using inhibiting antibodies. Klin Wschr 1976;54(8):357-360.
- 8 Hørder M, Elser RC, Gerhardt W, et al. IFCC methods for the measurement of catalytic concentration of enzymes. Provisional recommendation IFCC method for creatine kinase Appendix A. J Int Fed Clin Chem 1990;2:26-35.
- 9 Braun S, Rösenthaller F, Jarausch J, et al. Analyte Stability of CK-MB Activity and cTnT in ICU Patient Serum and Heparin Plasma. Poster presented at Medica 2004, Düsseldorf. (Roche Diagnostics GmbH No. 04587979990).
- 10 Wu AHB, Bowers GN. Evaluation and comparison of immunoinhibition and immunoprecipitation methods for differentiating MB from BB and macro forms of creatine kinase isoenzymes in patients and healthy individuals. Clin Chem 1982;28:2017-2021.
- 11 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.
- 12 Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.
- 13 Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.
- 14 Remaley AT, Wilding P. Macroenzymes: Biochemical Characterization, Clinical Significance, and Laboratory Detection. Clin Chem 1989;35:2261-2270.
- 15 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.
- 16 Klein G, Berger A, Bertholf R, et al. Abstract: Multicenter Evaluation of Liquid Reagents for CK, CK-MB and LDH with Determination of Reference Intervals on Hitachi Systems. Clin Chem 2001;47:Suppl. A30.
- 17 Thomas L, Müller M, Schumann G, et al. Consensus of DGKL and VDGH for interim reference intervals on enzymes in serum. J Lab Med 2005;29(5):301-308.
- 18 Stein W. Strategie der klinischen-chemischen Diagnostik des frischen Myokardinfarktes. Med Welt 1985;36:572-577.
- 19 Zawta B, Klein G, Bablok W. Temperature Conversion in Clinical Enzymology? Klin Lab 1994;40:33-42.
- 20 Myocardial Infarction Redefined - A Consensus Document of the Joint European Society of Cardiology/ American College of Cardiology Committee for the Redefinition of Myocardial Infarction. Eur Heart J 2000;21:1502-1513.
- 21 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

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

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

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.

COBAS, COBAS C, COBAS INTEGRA, PRECINORM, PRECIPATH and PRECICONTROL are trademarks of Roche.

All other product names and trademarks are the property of their respective owners.

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

© 2015, Roche Diagnostics



Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

Distribution in USA by:

Roche Diagnostics, Indianapolis, IN

US Customer Technical Support 1-800-428-2336

