BECKMAN COULTER

AU/DxC AU US

Instructions For Use

CKMB
CREATINE KINASE-MB (CK-MB)

REF OSR61155 2 x 22 mL R1-1, 2 x 4 mL R1-2, 2 x 6 mL R2

For *in vitro* diagnostic use only.

For Rx use only

PRINCIPLE

INTENDED USE

System reagent for the quantitative determination of Creatine Kinase-MB isoenzyme in human serum and plasma on Beckman Coulter AU/DxC AU analyzers.

SUMMARY AND EXPLANATION

Measurements of Creatine Kinase (EC 2.7.3.2) are used in the diagnosis and treatment of myocardial infarction and muscle disease, such as progressive Duchenne-type muscular dystrophy.

Creatine Kinase is a dimeric enzyme composed of M and/or B subunits which associate to form the CK-MM, CK-MB and CK-BB isoenzymes.[1,2] Following myocardial infarction the CK-MM level rises and reaches a peak between 18-30 hrs, the increase being similar to that of the total CK activity. CK-MB also rises following MI, however, it reaches a peak up to 12 hrs earlier than CK-MM making it an important early indicator of MI. The use of Total CK and CK-MB in the diagnosis of MI is the most important single application of CK measurements in clinical chemistry.[1,3]

METHODOLOGY

This CK procedure is a modification of the IFCC method[4,5] The R1 reagent contains an antibody which binds to the M subunit of CK in the serum sample thereby inhibiting the activity of the M subunit. The B subunit of the enzyme remains free to act on the substrate present in the R2 reagent. CK reversibly catalyzes the transfer of a phosphate group from creatine phosphate to adenosine diphosphate (ADP) to give creatine and adenosine triphosphate (ATP) as products. The ATP formed is used to produce glucose-6-phosphate and ADP from glucose. This reaction is catalyzed by hexokinase (HK) which requires magnesium ions for maximum activity. The glucose-6-phosphate is oxidized by the action of the enzyme glucose-6-phosphate dehydrogenase (G6P-DH) with simultaneous reduction of the coenzyme nicotinamide adenine dinucleotide phosphate (NADP) to give NADPH and 6-phosphogluconate. The rate of increase of absorbance at 340/660 nm due to the formation of NADPH is directly proportional to the activity of CK-MB in the sample.

$$\text{Creatine Phosphate} + \text{ADP} \xrightarrow{\text{CK}} \text{Creatine} + \text{ATP}$$

$$\text{ATP} + \text{Glucose} \xrightarrow{\text{HK},Mg2^{+}} \text{ADP} + \text{Glucose-6-phosphate (G-6-P)}$$

$$\text{G-6-P} + \text{NADP}^{+} \xrightarrow{\text{G6P-DH}} \text{6-phosphogluconate} + \text{NADPH} + \text{H}^{+}$$

SPECIMEN

SPECIMEN STORAGE AND STABILITY

Protect samples from light for maximum stability. CK-MB is stable in serum/plasma for 2 days when stored at 20 - 25°C, 7 days when stored at 4 - 8°C and up to 1 year when stored at -20°C. [6]

Specimen storage and stability information provides guidance to the laboratory. Based on specific needs, each laboratory may establish alternative storage and stability information according to good laboratory practice or from alternative reference documentation.

Additional handling conditions as designated by this laboratory:

SPECIMEN COLLECTION AND PREPARATION

Serum and Na/Li heparinized plasma samples free from hemolysis are the recommended specimens. Allow specimen to clot. Remove serum from cells promptly to minimize hemolysis and contamination by adenylate kinase from the red cells. Plasma samples may occasionally produce unpredictable rate reactions resulting in false low results.[4] Plasma with EDTA, oxalate or citrate is not recommended.

Additional instructions for patient sample preparation as designated by this laboratory:

Additional type conditions as designated by this laboratory:

REAGENTS

CONTENTS

CK-MB Reagent.

Reagent storage location in this laboratory:

WARNING AND PRECAUTIONS

1. Exercise the normal precautions required for handling all laboratory reagents.
2. Dispose of all waste material in accordance with local guidelines.
3. This product contains material of animal origin. The product should be considered as potentially capable of transmitting infectious diseases.

REACTIVE INGREDIENTS

Final concentration of reactive ingredients:

Imidazole (pH 6.7)	100 mmol/L
Hexokinase (HK)	≥4 kU/L (41.67 µkat/L)
NADP	2.0 mmol/L
G6P-DH	≥2.8 kU/L (25 µkat/L)
ADP	2.0 mmol/L
Mg-Acetate	10 mmol/L
AMP	5.0 mmol/L
Diadenosine pentaphosphate	10 µmol/L
EDTA	2.0 mmol/L
Glucose	20 mmol/L
Creatine Phosphate	30 mmol/L
N-Acetylcysteine	0.2 mmol/L
Activator	26 mmol/L
Antibody to CK-M subunit	Variable
Also contains preservatives.	

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

CK-MB R1-1	DANGER	
		
	H316	Causes mild skin irritation.
	H360	May damage fertility or the unborn child.
	P201	Obtain special instructions before use.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P308+P313	IF exposed or concerned: Get medical advice/attention.
	P332+P313	If skin irritation occurs: Get medical advice/attention.
		Imidazole 0.1 - < 1%
CK-MB R1-2	DANGER	
		
	H316	Causes mild skin irritation.
	H360	May damage fertility or the unborn child.
	P201	Obtain special instructions before use.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P308+P313	IF exposed or concerned: Get medical advice/attention.
	P332+P313	If skin irritation occurs: Get medical advice/attention.
		Imidazole 0.1 - < 1%
		Thioglycerol 1 - 5%
CK-MB R2	WARNING	
		
	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

EQUIPMENT AND MATERIALS

For use on the AU480, AU680, AU5800, DxC 500 AU, DxC 500i and DxC 700 AU Beckman Coulter Analyzers.

Storage location of test tubes or sample cups in this laboratory:

REAGENT PREPARATION

R1: Ensure complete transfer of R1-2 into R1-1 by pouring an aliquot of R1-1 buffer into R1-2, mix gently, then transfer entire contents back into R1-1. Mix by gentle inversion before placing on board the instrument.
R2: The reagent is ready for use and can be placed directly on board the instrument. No preparation is required.

STORAGE AND STABILITY

1. The unopened reagents are stable until the expiration date printed on the label when stored at 2 – 8°C.
2. Opened reagents are stable for 30 days when stored in the refrigerated compartment of the analyzer.

INDICATIONS OF DETERIORATION

Visible signs of microbial growth, gross turbidity or precipitate, or any change in reagent color may indicate degradation and warrant discontinuance of use.

Additional storage requirements as designated by this laboratory:

STABILITY OF FINAL REACTION MIXTURE

The Beckman Coulter AU/DxC AU analyzer automatically computes every determination at the same time interval.

CALIBRATION

CALIBRATION INFORMATION

Calibration of this CK-MB procedure is based upon the theoretical extinction coefficient for NADPH, which has a molar absorptivity of 6300 at 340/660 nm.

QUALITY CONTROL

During operation of the Beckman Coulter AU/DxC AU analyzer, at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed after calibration with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate Beckman Coulter

AU/DxC AU analyzer Instructions For Use (IFU) and Reference Manual. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Location of controls used at this laboratory.

CONTROL NAME	SAMPLE TYPE	STORAGE

TESTING PROCEDURE(S)

A complete list of test parameters and operational procedures are provided in the relevant AU/DxC AU analyzer IFU and Reference Manual.

RESULTS INTERPRETATION

The default unit of measure is U/L, for conversion to SI units (µkat/L) the result is divided by 60.

REPORTING RESULTS

EXPECTED RESULTS

Serum:[7] <24 U/L

Reference Intervals shown above were taken from the literature. Expected values may vary with age, sex, sample type, diet and geographical location. Each laboratory should verify the transferability of the expected values to its own population, and if necessary determine its own reference interval according to good laboratory practice. For diagnostic purposes, results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

Expected reference ranges in this laboratory:

INTERVALS	SAMPLE TYPE	UNITS

Additional reporting information as designated by this laboratory:

PROCEDURAL NOTES

INTERFERENCES

Results of studies[8] show that the following substances interfere with this Creatine Kinase-MB assay.

The criteria for no significant interference is recovery within 10% of the initial value

Bilirubin:	No significant interference up to 40 mg/dL Bilirubin
Hemolysis:*	
Lipemia:	No significant interference up to 400 mg/dL Intralipid**

*Adenylate Kinase from red blood cells may react with the reagent to produce spurious results and such specimens should not be used.

**Intralipid, manufactured by KabiVitrium Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

In very rare cases gammopathy, especially monoclonal IgM (Waldenström's macroglobulinemia), may cause unreliable results.

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc., makes no representation about the completeness or accuracy of results generated by future studies. Further information on interfering substances is available.[9]

Laboratory specific procedure notes:

PERFORMANCE CHARACTERISTICS

PERFORMANCE CHARACTERISTICS

Data contained within this section is representative of performance on Beckman Coulter systems. Data obtained in your laboratory may differ from these values.

DYNAMIC RANGE / ANALYTICAL MEASURING RANGE

The CK-MB procedure is linear from 10 to 2,000 U/L. Samples exceeding the upper limit of linearity should be diluted and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically utilizing the AUTO REPEAT RUN.

Note: For inhibition of adenylate kinase the recommended inhibitors AMP/Ap5A are included, but as the inhibition can never be completely 100% a residual activity could affect low CK-MB activity results.

Inhibition capacity of the anti-CK-M antibody is > 99% at a CK-MM level of 8,000 U/L. In samples where the total CK activity exceeds 8,000 U/L, CK-MB should be measured using a pre-diluted sample to ensure adequate inhibition of CK-M.

SENSITIVITY

Typical change in absorbance per minute for 1 U/L of CK-MB is approximately 0.12 mAbsorbance.

Limit of Quantitation

The Limit of Quantitiation (LOQ) using serum settings for the CK-MB reagent was determined to be 4 U/L. The Limit of Quantitiation (LOQ) using serum settings for the CK-MB reagent on the AU5800 was determined to be 9.42 U/L. The limit of Quantitation (LOQ) using serum settings for the CK-MB reagent on the DxC 700 AU was determined to be 6.25 U/L. This was determined according to CLSI protocol EP17-A[10] and represents the lowest concentration of CK-MB that can be measured with a total imprecision of 20%.

Note

Macro CK is an atypical form of CK that is composed of immunoglobulin complexes of normal isoenzymes. It migrates electrophoretically between MM & MB and is found mainly in elderly women. It is of no clinical significance, but its presence may cause falsely elevated results. If Macro-CK contribution is suspected its presence should be confirmed by electrophoresis.[11]

In very rare cases, gammopathy, especially monoclonal IgM (Waldenström's macroglobulinemia) can cause unreliable results.

METHODS COMPARISON

Reference[12]

Patient serum samples were evaluated in method comparison studies.

Results of Deming regression analysis were as follows:

Y Method	DxC 500 AU
X Method	DxC 700 AU
Slope	0.964
Intercept	-0.4005
Correlation Coeff. (r)	0.9996
No. of Samples (n)	104
Range (U/L)	13.314 - 1800.404

PRECISION

Reference[12]

Estimates of precision, based on CLSI recommendations,[13] are consistent with typical performance. The within run precision is less than 5% CV or SD ≤ 2.3 U/L and total precision is less than 6.5% CV or SD ≤ 3 U/L. Assays of control sera were carried out and data reduced following CLSI guidelines above.

N = 80	Within run		Total	
Mean, U/L	SD	CV%	SD	CV%
21	0.65	3.2	1.63	7.9
111	0.86	0.8	2.15	1.9
279	1.57	0.6	3.76	1.3

ADDITIONAL INFORMATION

DxC 700 AU analyzers require that each reagent application has a standard format of abbreviated Test Name. This Test Name is required to allow automated loading of the calibrator information for each application. Refer to the table below for the Test Name assigned to each application for this assay.

Test Name	Description
CKM1U	CK-MB (Serum)

Refer to the Beckman Coulter Chemistry Systems Reagent Guide (BAGUIDE) for specific chemistry information for the AU/DxC AU clinical chemistry systems and guidance on symbols used on all AU/DxC AU product labelling.

Setting Sheet Footnotes

User defined

* Values set for working in U/L. To work in SI units (µkat/L) divide by 60.

AU5800: Ψ Parameters specific factors provided by service engineer for each ring

Ψ System Factor

REVISION HISTORY

Add DxC 500i instrument to IFU

Preceding version revision history

Updated Specimen Section

Updated REPORTING RESULTS section

Updated PROCEDURAL NOTES section

Updated Performance Characteristics section

Updated References section

REFERENCES

1. Tietz, N.W., Fundamentals of Clinical Chemistry. 3rd Edition, (1987), 383pp.
2. Christenson, R.H., Ohman, E.M., Topol, E.J., Hanesian, M.A., et al. Creatine Kinase MM and MB Isoforms in Patients Receiving Thrombolytic Therapy and Acute Angiography. Clin Chem 1995; 41(6) 844-852.
3. Adams, J.E., Abendschein, D.R., Jaffe A.S., Biochemical markers of myocardial injury: Is MB creatine kinase the choice for the 1990s. Circulation 1993; 88: 750-63.
4. Horder M., Elsner R., et al., Approved Recommendation of IFCC Methods for the Measurement of Catalytic Concentration of Enzymes, Part 7 IFCC Method for Creatine Kinase, Appendix A, J. Clin. Chem. Clin. Biochem. 29, 435, 1991.
5. Szasz, G., Gerhardt, W. and Gruber, W., Clin Chem, 23: 1888, 1977.
6. Ehret W, Heil W, Schmitt Y, Topfer G, Wisser H, Zawta B, et al. Use of anticoagulants in diagnostic laboratory investigations and stability of blood, plasma and serum samples. WHO/DIL/LAB/99.1 Rev.2:28pp.
7. Kairisto V, Hänninen KP, Leino A, Pulkki K, Peltola O, Näntö V et al. Generation of reference values for cardiac enzymes from hospital admission laboratory data. Eur J Clin Chem Clin Biochem 1994;32:789-96.
8. CLSI EP07-ED3:2018 Interference Testing in Clinical Chemistry, 3rd Edition.
9. AACC Effects on Clinical Laboratory Tests: Drugs, Disease, Herbs and Natural Products https://clinfx.wiley.com/aaccweb/aacc/
10. Tholen DW, Linnet K, Kondratovich M, Armbruster DA, Garrett PE, Jones RL, et al Protocols for determination of limits of detection and limits of quantitation; approved guideline. NCCLS Document EP17-A. NCCLS, Pennsylvania, USA, 2004
11. Jacobs, C., Kasten, B., Dermott, W., Wolfson, W. The Laboratory Test Handbook, 2nd Edition. Stow/Ohio, Lexi-comp/Mosby, 1988.
12. Data is on file for specific AU/DxC AU analyzers.
13. CLSI EP05-A3: 2014 Evaluation of Precision of Quantitative Measurement Procedures.

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