TECO DIAGNOSTICS
1268 N. Lakeview Ave.
Anaheim, CA 92807
1-800-222-9880

CREATINE KINASE – MB (CK-MB) TC MATRIX-160

INTENDED USE

This test is an in-vitro test used in clinical laboratories for the quantitative immunological determination of myocardial-brain (MB) subunit's catalytic activity of creatine kinase (CK) enzyme in human serum and plasma by means of analyzers.

INTRODUCTION

Creatine Kinase enzyme has a dimeric structure consists of two subunits, each of which is almost 40 kDa. As the active form of enzyme is dimeric, there are isoenzymes containing only three different double subunits named CK-BB (CK-1), CK-MB (CK-2) and CK-MM(CK-3).

CK enzyme activity is mostly in striated muscles and heart tissue. While the other tissues such as brain smooth muscles, gastrointestinal system and bladder smooth muscles contain much less activity, the liver and erythrocytes actually lack activity.[1]

More than 98% of the substance of total CK in the skeletal muscle consists of CK-MM, and less than 2% is CK-MB; however, it may increase up to 5-15% in people who train or have muscle disease. 70-80% of the total CK content of the heart muscle is CK-MM and the remaining 20-30% is CK-MB. As a general rule, the heart muscle is the only tissue containing more than 5% CK-MB.

Other organs such as brain have less CK activity and a large part of this activity usually consists of CK-BB. Yet CK-BB is rarely seen in the plasma and has no diagnostic significance. More than 95% of the plasma total CK content in a healthy individual belongs to CK-MM [2]

PRINCIPLE

Immunochemical, UV measurement

Sample to be measured for CK is incubated with mouse derived monoclonal antibodies specific to CK-M subunit. In this way, CK-M activity in the sample is substantially inhibited without the activity of CK-B subunit being affected. Rest of the activity of CK-B subunit determined by total CK measurement method is equal to the half CK-MB activity, since there is almost no CK-BB activity in serum, and the catalytic activities of serum CK-M and CK- B subunits are the same. In this case, CK-MB activity is obtained by multiplying the result by 2. The logic of total CK measurement method is based on 3 enzymatic reaction as stated below:

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

$$\text{ATP + Glucose} \xrightarrow{HK} \text{ADP + Glucose – 6 – Phosphate}$$

$$\text{Glucose 6 – Phosphate + NADP}^{+} \xrightarrow{G6PDH} \text{6- Phosphogluconate + NAPH}$$

According to this method, CK enzyme catalyses ADP phosphorylation reversibly in the presence of creatine phosphate to create ATP and creatine. The ATP formed in this reaction phosphorylates the glucose molecule with the reaction catalyzed by the hexokinase (HK) enzyme, forming glucose-6-phosphate (G-6-P) and ADP products. In the last step reaction, Glucose-6-phosphate dehydrogenase (G6PDH) enzyme catalyzes the G-6-P molecule's oxidization to 6-phosphogluconate and NADP+ is reduced to NADPH during this reaction

The absorbance change which occurred during reaction and is related to this reduction is measured spectrophotometrically at 340 nm ultraviolet (UV) wavelength and is directly proportional to the total CK enzyme activity in the sample

REAGENT COMPONENTS

Reagent 1

Imidazole pH 6.7	≤ 132 mmol/L
Glucose	≤ 24 mmol/L
N-Acetylcystein	≤ 27.6 mmol/L
Magnesium acetate	≤ 12 mmol/L
EDTA	≤ 2.52 mmol/L
ADP	≤ 3 mmol/L
NADP	≤ 2.76 mmol/L
AMP	≤ 6 mmol/L
Diadenosinpentaphosphate	≤ 13.2 mmol/L
Glucose-6-Phosphate-Dehydrogenase	≥ 1.5 kU/L
Hexokinase	≥ 2.5 kU/L

Inhibits CK-MM (Human based creatine kinase MM subunit) with Anti CK-MM mouse based monoclonal Antibodies

Inhibiting capacity	≥ 200 U/L

Reagent 2

Creatine phosphate	≤ 233 mmol/L
Imidazole pH 6.7	≤ 132 mmol/L
Glucose	≤ 24 mmol/L
Magnesium acetate	≤ 12 mmol/L
EDTA	≤ 2.52 mmol/L

Material required but not provided

CK-MB Calibrator
Two levels of CK-MB Controls

REAGENT PREPARATION

Reagents are ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at 2-8°C until the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 30 days at 2-8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[3]

SAMPLE REQUIREMENTS

Serum and K_2-EDTA, K_3- EDTA plasma can be used and are collected according to the standard procedures. Multiple sample freezing and thawing should be avoided. The sample should be homogenized before testing.

CK-MB activity stability in serum and plasma:

Serum:[4]

8 hours at 20 - 25°C
8 days at 2 - 8°C
4 weeks at -20°C

Plasma:[5]
2 days at 20 - 25°C
7 days at 2 - 8°C
1 year at -20°C.

Unit Conversion:
CK-MB U/L x 0.0167 CK-MB µkat/L

CALIBRATION AND QUALITY CONTROL
Calibration: The assay requires the use of CK- MB Calibrator. Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used. Calibration stability is 30 days.

Control: Commercially available control material with established values determined by this method can be used.

Two levels of CK-MB control should be run every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS
Due to the wide tissue distribution of total CK, its measurement is not recommended in the routine diagnosis of acute MI. For more accurate clinical diagnosis of acute MI, total CK must be used together with a more sensitive biomarker such as cardiac troponin or CK-MB, as well as clinical findings and EKG.[6]
Determined CK and CK-MB medical decision level values in MI evaluation are as follows:[7]

CK-MB : < 25 U/L
CK Men : < 190 U/L
CK Women : < 170 U/L

The CK-MB activity accounts for 6-25% of the total CK activity. CK varies with physical activity level and race in healthy individuals.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range.

Reference interval has been verified by using Clinical and Laboratory Standards Institute (CLSI) EP28-A3c protocol.

PROCEDURE

Test Name:	CK-MB	R1:	120
Full Name:	CK-MB	R2:	26
Pri. Wave:	340 nm	Sample volume:	9.0
Sec. Wave:	660 nm	Calibration Type:	2 point linear
Assay/ Point:	Kinetic	K Value:	/
Start - End:	15 - 21	Point:	2
Decimal place:	1	Blank Type:	Water
Unit:	U/L	Point 0 (Blank) Con.:	0.0
Linearity Range:	2.000 - 1000.00	Point 1 (STD) Con.:	Standard/
Correlation Factor:	1.0000 - 0.0000		Calibrator

PERFORMANCE CHARACTERISTICS
Measuring Interval
According to CLSI EP34-ED1:2018, "Measuring Interval" refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte's lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[8]
The determined analytic measuring interval for CK-MB: 10- 1000 U/L.

Detection Capability
Limit of Detection (LoD): 7 U/L
Limit of Quantitation (LoQ): 10 U/L
Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[9]

Linearity: This method shows measurement linearity in the activities up to 1000 U/L.

Autoanaylzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For manual dilution procedure, dilute the sample 10-fold using 0.90% isotonic. After the dilution, multiply the result of rerun sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity studies data have been verified by using CLSI EP06-A:2003 protocol.[10]

Precision : Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatibility and Within- Laboratory Precision/Within-Device values have been obtained according to the running results.
According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[11]

Repeatability (Within Run) and Repeatability (Day to Day) SD and CV% values of CK-MB have been given in the table 1 and 2 respectively.

Table 1. CK-MB Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD*	CV%	n
28 U/L	0.80	2.86	80
85 U/L	1.03	1.21	80

**SD: Standard deviation*
Note : This working system has been named “Within-Run Precision” in the previous CLSI-EP05-A2 Manual. [12]

Table 2. CK-MB Repeatability (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD*	CV%	n
28 U/L	0.96	3.42	80
85 U/L	2.08	2.45	80

Note : This working system has been named “Within-Run Precision” in the previous CLSI-EP05-A2 Manual. [12]

Method Comparison
Correlation with a comparative method: r=0.98
According to Passing-Bablok equation:
Slope: 0.97
Intercept: 2.98

Interference
Endogenous interferant and analyte concentrations that have been used in the CK-MB scanning tests have been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals. [13,14]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from CK-MB interference scanning test is appropriate, is determined as ±10%.[15]

In CK-MB test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Interferent and Concentration	CK-MB Liquid Target (U/L)	N	Observed Recovery %
Bilirubin Total 60 mg/dL	50	*5	92
	80	*5	91
Triglyceride 1700 mg/dL	42	*5	93
	94	*5	94
Hemoglobin 500 mg/dL	50	*5	91
Hemoglobin 600 mg/dL	94	*5	92

** Total acceptable error rate determined as interference limit and repeatability (within run) pre-detected for the related method were used for the calculations of how many times the control and test samples prepared as a serum pool are going to be run repetitively. In the calculations, the accepted error rate for type 1 (a error) was 5% and for type 2 (ß error) was 10% (90% power). [14]*

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium floride, iodoacetate, hydrochloride acid) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some ssssmisjudgements.[14]

These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD: For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.
CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA standards.

REFERENCES :

1. Mauro Panteghini and Renze Bais. Chapter 29, Serum Enzymes p404-434 In: Tietz Textbook of Clinical Chemistry and Molecular Diagnostic 6th ed. St. Louis, Missouri: Copyright 2018 by Elseiver
2. Peter Rae et al. Chapter 12 Cardiovacsular Disorders; p167-182 In: Clinical Biochemistry Lecture Notes 10 th Edition published 2018 by John Wiley & Sons Ltd
3. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline. CLSI Document EP25-A. Wayne, PA: CLSI; 2009.
4. Braun S, Röschenthaler 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).
5. Use of Anticoagulants in Diagnostic Laboratory Investigations. WHO Publication WHO/DIL/LAB/99.1 Rev. 2: Jan 2002.
6. 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.
7. 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
8. Clinical and Laboratory Standards Institute (CLSI). Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking - First Edition. CLSI Document EP34. Wayne, PA: CLSI; 2018.
9. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline - Second Edition. CLSI Document EP17-A2. Wayne, PA: CLSI; 2012.
10. Clinical and Laboratory Standards Institute (CLSI). Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach First Edition. CLSI Document EP06-A. Wayne, PA: CLSI; 2003.
11. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition. CLSI Document EP05-A3. Wayne, PA: CLSI; 2014.
12. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition. CLSI Document EP05-A2. Wayne, PA: CLSI; 2004.
13. Clinical and Laboratory Standards Institute (CLSI). Supplemental Tables for Interference Testing in Clinical Chemistry First Edition. CLSI Document EP37. Wayne, PA: CLSI; 2018.
14. Clinical and Laboratory Standards Institute (CLSI). Interference Testing in Clinical Chemistry Third Edition. CLSI Document EP07. Wayne, PA: CLSI; 2018.
15. CLIA proficiency testing criteria for acceptable analytical performance, as printed in the Federal Register July 11, 2022;87(131:41194-242.

C614–TC1:10/2023

Manufactured by:

TECO DIAGNOSTICS
1268 N. LAKEVIEW AVE.
ANAHEIM, CA 92807
U.S.A.