

CK-MB (Creatine Kinase-MB)

Diagnostic reagent for determination of CK-MB concentration.

Liquid. Dual reagents. Store at +2/+8°C. For in Vitro Diagnostic Use (IVD). **Do not freeze.**

Ref No	Pack
MH-142	75 mL
MH-143	50 mL

Changes made in the instructions for use are marked as grey.

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 creatinine kinase (CK) enzyme in human serum and plasma by means of analyzers.

GENERAL INFORMATION

Creatinine Kinase enzyme has a dimeric structure consisting 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.

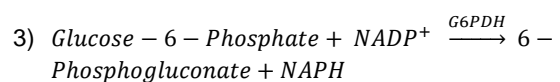
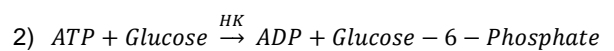
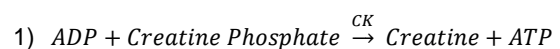
Other organs such as brain have less CK activity and a large part of this activity usually consists of CK-BB. Yet CK-BB is rarely seen in the plasma and has no diagnostic significance. More than 95% of the plasma total CK content in a healthy individual belongs to CK-MM²

TEST 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:



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 catalyses the G-6-P molecule's oxidation 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) wave length 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
Magnesiumacetate	≤ 12	mmol/L
EDTA	≤ 2,52	mmol/L
ADP	≤ 3	mmol/L
NADP	≤ 2,76	mmol/L
AMP	≤ 6	mmol/L
Diadenosinpentaphosphate	≤ 13.2	µmol/L
Glucose-6-Phosphate-Dehydrogenase	≥ 1.5	kU/L
Hexokinase	≥ 2.5	kU/L

Inhibits CK-MM (Human based creatinine kinase MM subunit) with Anti CK-MM mouse based monoclonal antibodies

inhibiting capacity ≥ 200 U/L

Reagent 2

Creatinephosphate	≤ 223	mmol/L
Imidazole pH 6.7	≤ 132	mmol/L
Glucose	≤ 24	mmol/L
Magnesiumacetate	≤ 12	mmol/L
EDTA	$\leq 2,52$	mmol/L

REAGENT PREPARATION

Reagents are ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8°C till 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 K₂-EDTA, K₃- 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 μ kat/L

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of a Validity CK-MB Calibrator. We recommend:

CK-MB Calibrator-Lyophilised

Ref.No: VT-024

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. We recommend:

CK-MB Control Level 1- Lyophilised

Ref.No: VT-022

CK-MB Control Level 2- Lyophilised

Ref.No: VT-023

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.

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) $\leq 20\%$.

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

Linearity

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

Autoanalyzer’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. Repeatability 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 “Total 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 has 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 $\pm 10\%$.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.

Interferant 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 (α error) was 5% and for type 2 (β 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 fluoride, iodoacetate, hydrochloride acids) 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 misjudgements.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.

Danger

EUH032 :Releases a very toxic gas if contacts with acid.

H317 :May cause allergic skin reaction.

Precaution

P280 :Use protective gloves / clothes / glasses / mask.

P264 :Wash your hands properly after using.

P272 :Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352 :Wash with plenty of water and soap if it contacts with skin.

P333+P313 :Seek medical help if it irritates your skin or develops rash.

P362+P364 :Remove contaminated clothes and wash properly before using.

Disposal

P501 :Dispose the vials and contents according to the local regulations.

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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
R2	Reagent 2
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests