CREATINE KINASE – MB (CK – MB)

Diagnostic reagent for the in-vitro quantitative determination of Creatine Kinase MB (CK-MB) in human serum on manual systems.

REF: V/MB05.005	25 test	REF: V/MB05.010	50 test

CLINICAL SIGNIFICANCE

Creatine kinase (CK) is an enzyme which is contained in heart, brain and skeletal muscles. Thus, an increase of circulating level of CK may be associated to myocardial infarction, acute cerebrovascular disease, trauma or diseases of skeletal muscles. After a myocardial infarct, CK level begins raising between 4th and 6th hour after first acute symptoms, reaching the peak between 18th and 30th hour and coming back to normal values during the 3rd day. CK is present in serum in dimeric forms as CK-MM, CK-MB, and CK- BB and as macro-enzymes. Measurement of CK-MB is a quite specific test for detection of cardiac muscle damage and is therefore used for diagnosis and monitoring of myocardial infarction

METHOD PRINCIPLE (2)

A specific antibody inhibits the M subunits of CK-MM and CK-MB, and thus allows determination of the B subunit of CK-MB (assuming the absence of CK-BB or CK-1). CK-B catalytic concentration, which corresponds to half of CK-MB concentration, is determined from the rate of NADPH formation, measured at 340 nm, by means of the hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6PDH) coupled reactions.

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

$$\text{ATP + Glucose} \xrightarrow{\text{HK}} \text{ADP + Glucose-6-phosphate}$$

$$\text{Glucose-6-phosphate+NADP+} \xrightarrow{\text{G6PDH}} \text{6-Phosphogluconate +NADPH+H+}$$

REAGENT COMPOSITION

Reagent (R1): Buffer / Coenzyme (pH 6.7)	
Imidazol	125mmol/L
D-Glucose	25mmol/L
N-Acetyl-L-Cysteine	25 mmol/L
Magnesium acetate	12.5mmol/L
NADP	2.5 mmol/L
EDTA	2 mmol/L
Reagent (R2): Enzymes	
ADP	15.2mmol/L
AMP	25 mmol/L
P1,P5-di (adenosine-5'-) penta-phosphate	103 mmol/L
Glucose-6-phosphate Dehydrogenase (G6PDH)	9 KU/L
Creatine phosphate	250 mmol/L
Hexokinase (HK)	3 KU/L
Anti-human-CK-M.	2000 U/L

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the **Lab.Vie**. CK-MB reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

Lab.Vie. CK-MB working solution is prepared by combining four volumes of R1 with one volume of R2 and mix gently, e.g.4.0 ml R1 +1.0 ml R2. CK-MB working solution is stable for 2 weeks at 2-8°C away from light sources. **Lab.Vie**. CK-MB reagents are stable up to the expiry date labeled on the bottles when properly stored refrigerated at 2–8°C.

Deterioration
The **Lab.Vie**. CK-MB reagent is normally clear when protected from light and contaminations prevented during their use. Do not use **Lab.Vie**. CK-MB reagent if it is turbid or presence of foreign particles.

SPECIMEN COLLECTION AND PRESERVATION (3)

Serum or plasma
Specimen should be promptly separated from cells after blood collection, serum free of hemolysis is the preferred specimen. The plasma containing heparin, EDTA, citrate or fluoride may produce unpredictable reaction rates. The stability of CK-MB in serum is 2 hours at 20-25°C and 5 days at 2-8°C. If the Total CK-MB concentration in the sample is higher than 1000 U/L. Dilute the serum 1:2 with NaCl (150 mmol/L).

SYSTEM PARAMETERS

Wavelength	340 nm (334 – 365 nm)
Optical path	1 cm
Assay type	Kinetic
Direction	Increase
Sample Reagent Ratio e.g.: Reagent volume Sample volume	1:25 1 ml 40 µl
Temperature	37 °C
Equilibration time	60 Seconds
Read Time Reading interval No. of Reading	1 to 5 minutes 1 minute 6
Zero adjustment	against air
Sensitivity	2 U/L
Linearity	2000 U/L

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes, automatic pipet and Thermostatized cuvette
- Centrifuge and spectrophotometer

ASSAY PROCEDURE

	macro	Semi-micro
Reagent R1	800 µl	400 µl
Reagent R2	200 µl	100 µl
Mix well and incubate for 5 minute at 37°C.		
Specimen	40 µl	20 µl

Read initial absorbance after 60 seconds, and start timer immediately. Read again after 1, 2,3,4 and 5 minutes and determine the mean absorbance change per minute (ΔA/min).

CALCULATION

CK-MB U/L = ΔA/min x 8254

NB.: One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions. The concentration is expressed in units per liter of sample (U/L).

QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control serum of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact **Lab.Vie**. technical support.

PERFORMANCE CHARACTERISTICS

Precision	Within run (Repeatability)		Run to run (Reproducibility)	
	Normal level	High level	Normal level	High level
n	20	20	20	20
Mean U/L	45	129	40	130
SD. U/L	3.5	3.2	2.8	2.3
CV. %	3.8	1.5	4.7	1.5

The results of the performance characteristics depend on the analyzer used.

Accuracy (Methods Comparison)
Result obtained from **Lab.Vie**. CK-MB reagent compared with commercial reagent of the same methodology performed on 20 human sera give a correlation of 0.959.
Sensitivity
When run as recommended, the minimum detection limit of the assay is 2.0 U/L.
Linearity
The reaction is linear up to **Lab.Vie**. CK-MB concentration of 2000 U/l; specimens showing higher concentration should be diluted 1+2 using physiological saline and repeat the assay (result×3).

INTERFERING SUBSTANCES (2,4)

Haemolysis
No significant interference from haemoglobin up to < 2.5 g/L.
Icterus
No significant interference from free and conjugated bilirubin up to levels < 25 mg/dL.
lipemia
No significant interference from Lipids up to levels < 900 mg/dL.
Other drugs and substances may interfere.

Others
Presences in the sample of above normal concentrations of CK-BB or adenylate kinase, and of macro or mitochondrial CK interfere.

EXPECTED VALUES (3)

Serum and plasma	At 30°C	At 37°C
CK-MB	< 16 U/L	< 25 U/L
CK-MB/CK%	CK-MB ratio of 25U/L is consistent with actuate myocardial infarction. In case of suspicion of myocardial infarction, CK-MB values return into normal within 48 hours.	

DYNAMIC RANGE

< 25 U/L at 37°C; It is recommended that each laboratory should establish its own reference range.

REFERENCES

1. Tietz N. W., Textbook of Clinical Chemistry, 3rd edition. Burtis CA, Ashwood ER. WB Saunders Co., 1999 p 664-667, 1185-1190.
2. Friedman and Young. Effects of disease on clinical laboratory tests, 3th ed. AACC Press, 1997.
3. Tietz N. W., Clinical Guide to Laboratory Tests, 4th Edition, (2006) p 310-315.
4. Urdal P and Landaas S. Clin Chem 1979; 25: 461-465.

SYMBOLS IN PRODUCT LABELLING	
IVD For in-vitro diagnostic use	Σ Number of <n> test in the pack
LOT Batch Code/Lot number	Caution
REF Catalogue Number	Do not use if package is damaged
Temperature Limitation	Consult Instruction for use
Expiration Date	
Manufactured by	