Atlas Medical

Quantitative determination of creatine kinase - MB (CK-MB)

CE

IVD *For in -vitro diagnostic and professional use only*

2°C 8°C Store at 2-8°C

INTENDED USE

For quantitative determination of creatine kinase in human serum or plasma.

INTRODUCTION

Creatine kinase-MB (CK-MB) is a form of an enzyme found primarily in heart muscle cells. This test measures CK-MB in the blood.

CK-MB is one of three forms (isoenzymes) of the enzyme creatine kinase (CK).

Test results may vary depending on your age, gender, health history, the method used for the test, and other things. Your test results may not mean you have a problem. Ask your healthcare provider what your test results mean for you.

Levels of CK-MB do not rise in your blood within the first 4 to 6 hours after a heart attack. You may need to have repeated tests to see if you've had a heart attack.

Higher levels of CK-MB may mean that you have had a heart attack or have other heart problems. These include: Myocarditis, an infection and inflammation of the heart muscle Pericarditis, an infection and inflammation of the thin sac that surrounds the heart Cardiac defibrillation, when an electric shock is used to fix the heart rhythm. Higher levels of CK-MB may also mean more of the heart was damaged in the attack. Higher levels may also be caused by muscle damage elsewhere in your body, by diseases that affect your muscles, and by trauma to your chest.

PRINCIPLE OF THE METHOD

An antibody to the anti CK-M inhibits completely CK-MM and subunit (M) of the CK-MB. The activity of the non-inhibited CK-B subunit is then assayed by the following series of reactions:

$$\text{Phosphocreatine} + \text{ADP} \xrightarrow{\text{CK}} \text{Creatine} + \text{ATP}.$$

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

$$\text{G6P} + \text{NADP}^+ \xrightarrow{\text{G6P-DH}} \text{6-Phosphogluconate} + \text{NADPH} + \text{H}^+.$$

The rate of NADPH formation, measured photometrically, is proportional to the catalytic concentration of CK-B present in the sample.

REAGENTS

R 1 Buffer	Imidazol pH 6.7	100 mmol/L
	Glucose	20 mmol/L
	Magnesium acetate	10 mmol/L
	EDTA	2 mmol/L
R 2 Anti CK - MB Immunoinhibition kinetic Tablets	*Anti CK-M	2000 U/L
	ADP	2 mmol/L
	AMP	5 mmol/L
	di-Adenosine-5-pentaphosphate	10 mmol/L
	$NADP^+$	2 mmol/L
	Hexoquinase (HK)	2500 U/L
	Glucosa-6-phosphate deshydrogenase	1500 U/L
	N-acetylcysteine	20 mmol/L
	Creatinine phosphate	30 mmol/L
CK-Nac / CK-MB CONTROL (Optional)	Lyophilized human serum.	

*Anti CK-M sufficient to inhibit up to 2000 U/L of CK-MM.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 340 nm.
- Thermostatic bath at 25°C/ 30°C/ 37° C (± 0.1°C).
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

PRECAUTIONS

- CK-Nac / CK-MB CONTROL, Components from human origin have been tested and found to be negative for the presence of HBsAg, HCV, and antibody to HIV (1/2). However handle cautiously as potentially infectious.
- R1: H360 May damage fertility or the unborn child.
- Follow the precautionary statements given in MSDS and label of the product.

PREPARATION

Working reagent (WR):

- Dissolve one tablet of R 2 in one vial of R 1. Cap vial and mix gently to dissolve contents. Stability: 8 days at 2-8°C or 24 hours at 15-25°C.

STORAGE AND STABILITY

- All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented.
- Do not use the tablets if appears broken.
- Do not use reagents over the expiration date.
- Signs of reagent deterioration:
  - Presence of particles and turbidity.
  - Blank absorbance (A) at 340 nm $\geq$ 1.60.

SAMPLES

Serum or plasma: Stability 7 days at 2-8°C, protected from light.

CK-MB activity decreases a 10% after 24 hours at 4°C or 1 hour at 25°C.

PROCEDURE

1. Assay conditions:
   Wavelength: ……340 nm
   Cuvette:……1 cm light path
   Constant temperature ……25°C / 30°C / 37°C
2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a cuvette:

WR(mL)	1.0
Sample (µL)	40

4. Mix then Incubate for 10 minute.
5. Read initial absorbance (A) of the sample, start the stopwatch and read again after 5 minutes

(A_2).

6. Calculate the difference between absorbances : $\Delta A = A_2 - A_1$.

CALCULATIONS

ΔA x 825 = U/L de CK-B ΔA x 1651 = U/L de CK-MB

Units: 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).

Percentage of CK-MB activity in sample:

CK-MB Activity x 100 = % CK-MB Activity
CK Total Activity

Temperature conversion factors

To correct results to other temperatures multiply by:

Assay temperature	Conversion factor to		
	25°C	30°C	37°C
25°C	1.00	1.53	2.38
30°C	0.65	1.00	1.56
37°C	0.42	0.64	1.00

QUALITY CONTROL

- Control sera are recommended to monitor the performance of assay procedures.
- If control values are found outside the defined range, check the instrument, reagents and technique for problems.
- Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES

Heart infarct probability is high at the following conditions:

	25°C	30°C	37°C
CK-MB	> 10 U/L	> 15 U/L	> 24 U/L

CK-MB activity is between 6 and 25% of total CK activity.
These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

1. Measuring range:

Detection limit: 3,11 U/L.

2. Linearity:

The total CK activity must be determined by the CK-NAC activated method prior to the CK-MB assay. If the CK activity exceeds 1000 U/L, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

3. Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (U/L)	54.2	138.3	55.7	141.6
SD	1.45	1.33	1.62	1.39
CV (%)	2.67	0.96	2.92	0.98

4. Sensitivity:

1 U/L = 0,00029 ΔA / min.

5. Accuracy:

Results obtained using reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained using 50 samples were the following: Regression coefficient $(r)^2$: 0,996.
Equation of the regression line: y = 0,9919x - 0,1042.

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

INTERFERENCES

Hemolysis interferes with the assay.
A list of drugs and other interfering substances with CK-MB determination has been reported by young.

REFERENCES

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REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry