(CK-MB)

Quantitative determination of creatine kinase MB (CK-MB)

Store at 2-8°C

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:

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

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

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

The rate of NADPH formation, measured photometrically, is proportional to the catalytic concentration of CK-B present in the sample[1,2].

CLINICAL SIGNIFICANCE

CK-MB is an enzyme formed by the association of two subunits from muscle (M) and nerve cells (B). CK-MB is usually present in serum at low concentration; it is increases after an acute infarct of myocardium and later descends at normal levels. Also is increased, rarely, in skeletal muscle damage.
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

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	*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
	Hexokinase (HK)	2500 U/L
	Glucosa-6-phosphate deshydrogenase	1500 U/L
	N-acetylcysteine	20 mmol/L
	Creatinine phosphate	30 mmol/L

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

Optiona

CK-Nac / CK-MB CONTROL Lyophilized human serum.

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.

PREPARATION

– Working reagent (WR)
Dissolve one tablet of R 2 in 2.5 ml 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. CK-Nac / CK-MB CONTROL.

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 ≥ 1.60.

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.

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. 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 fimol of substrate per minute, in standard conditions. The concentration is expressed in units per litre of sample (U/L).

Percentage of CK-MB activity in sample:

$$\frac{\text{CK-MB Activity}}{\text{CK Total Activity}} \times 100 = \%\ \text{CK-MB Activity}$$

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

Measuring range: From detection limit of 2.85 U/L to linearity limit of 1000 U/L
If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.
Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (U/L)	138	9.6	141	8.85
SD	1.33	0.88	1.39	0.67
CV (%)	0.96	9.19	0.98	7.57

Sensitivity: 1 U/L = 0,00013 ΔA / min.
Accuracy: Results obtained using Atlas reagents (y) did not show systematic differences when compared with other commercial reagents (x). The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Hemolysis interferes with the assay[2].
A list of drugs and other interfering substances with CK-MB determination has been reported by Young et.al.

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