Creatine kinase - MB

Immunoinhibition. Kinetic UV

Quantitative determination of creatine kinase MB (CK-MB)

CE IVD

PACKAGING

Ref.: 101-0375	Cont.: 19 x 2.5 mL

Store at 2-8º C

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[5,6].

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

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].

REAGENTS

R 1 Buffer	Imidazol pH 6.7 Glucose Magnesium acetate EDTA		100 mmol/L 20 mmol/L 10 mmol/L 2 mmol/L
R 2 Anti CK-M	*Anti CK-M ADP AMP di-Adenosine-5- pentaphosphate $NADP^{+}$ Hexoquinase (HK) Glucosa-6-phosphate deshydrogenase N-acetylcysteine Creatinine phosphate		2000 U/L 2 mmol/L 5 mmol/L 10 mmol/L 2 mmol/L 2500 U/L 1500 U/L 20 mmol/L 30 mmol/L
Control Level 1	Ref.: 101-0697	1 x 2 mL	Lyophilized human control serum

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

PRECAUTIONS

R1: H360- May damage fertility or the unborn child.

Follow the precautionary statements given in MSDS and label of the product.

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 one vial of R 1.

Cap vial and mix gently to dissolve contents.

Stability: 8 days at 2-8º C or 24 hours at 15-25º C.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8º 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 25º C, 30º C or 37° C (± 0.1º C).
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

Serum or plasma[1]: Stability 7 days at 2-8º C, protected from light.

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

PROCEDURE

Notes: CHRONOLAB SYSTEMS has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

1. Assay conditions:
   Wavelength: . . . . . 340 nm
   Cuvette: . . . . . 1 cm light path
   Constant temperature . . . . . .25º C / 30º C / 37º 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 CK-B ΔA x 1651 = U/L CK-MB

Calculating factor in automatic analyzers by kinetic method (ΔA/min.) is 8255 of 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 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		
	25º C	30º C	37 ºC
25º C	1.00	1.53	2.38
30º C	0.65	1.00	1.56
37º C	0.42	0.64	1.00

QUALITY CONTROL

If control values are found outside the defined range, check the instrument, reagents and technique for problems CK-NAC/CK-MB CONTROL (L2) (Ref. 101-0762).

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:

	25º C	30º C	37º 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: Detection limit: 3.11 U/L.

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.

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

Sensitivity: 1 U/L = 0.00029 ΔA / min.

Accuracy: Results obtained using CHRONOLAB 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[2].

A list of drugs and other interfering substances with CK-MB determination has been reported by Young et. al[3,4].

BIBLIOGRAPHY

1. Abbot B et al. Creatinine kinase. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984: 1112-116.
2. Gerhardt W. et al. Creatine kinase B-Subunit activity in serum after immunohinhibition of M-Subunit activity. Clin Chem 1979;(25/7): 1274-1280.
3. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
4. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
5. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
6. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.

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