CK MB (NAC act.) KIT

(Immunoinhibition / Mod. IFCC method)

For the determination of CK - MB activity in serum.

(For Invitro Diagnostic Use Only)

Summary

CK is dimeric molecule composed of M and B subunits, which are immunologically distinct. It exists as three main iso enzymes CK - MM, CK - MB, and CK - BB. The CK - MM is found in the muscle while CK - MB is found mainly in the myocardial cells. The CK - BB is found mainly in the brain and lungs, and enters the blood stream only on injury to these organs like cerebrovascular accident or pulmonary infarction. Normally CK - MM is found in the blood. CK - MB levels increase significantly 4 - 6 hours following a myocardial infarction and peak at around 12 to 24 hours after the infarct. The levels return to normal, in case of no further myocardial damage, after 24 to 48 hours. Hence the increased levels of CK - MB along with elevated levels of total CK is a good indicator of myocardial infarction. CK - MB levels usually do not rise in chest pain caused by angina, pulmonary embolism or congestive heart failure.

Principle

CK - M fractions of the CK - MM and the CK - MB in the sample are completely inhibited by an anti CK - M antibody present in the reagent. Then the activity of the CK - B fraction is measured by the CK (NAC act.) method. The CK - MB activity is obtained by multiplying the CK - B activity by two.

Normal reference values

Serum = upto 24 U/L at 37°C

Indication of myocardial infarction is based on the following factors:

Total CK (male) : > 195 U/L at 37°C

(female) : > 170 U/L at 37°C

CK - MB : > 24 U/L at 37°C

CK - MB to Total CK : > 6% - 25%

It is recommended that each laboratory establish its own normal range representing its patient population.

Contents	**2 x 10 ml**	**2 x 25 ml**	**2 x 75 ml**
L1 : Enzyme Reagent	2 x 8 ml	2 x 20 ml	2 x 60 ml
L2 : Starter Reagent	2 x 2 ml	2 x 5 ml	2 x 15 ml

Storage / stability

Contents are stable at 2-8°C till the expiry mentioned on the labels.

Reagent Preparation

Reagents are ready to use.

Working reagent : For sample start assays a single reagent is required. Pour the contents of 1 bottle of L2 (Starter Reagent) into 1 bottle of L1 (Enzyme Reagent). This working reagent is stable for at least 10 days when stored at 2-8°C.

Alternatively for flexibility as much of working reagent may be made as and when desired by mixing together 4 parts of L1 (Enzyme Reagent) and 1 part of L2 (Starter Reagent).

Alternatively 0.8 ml of L1 and 0.2 ml of L2 may also be used instead of 1 ml of the working reagent directly during the assay.

Sample material

Serum. Free from hemolysis.

Procedure

Wavelength / filter : 340 nm

Temperature : 37°C / 30°C / 25°C

Light path : 1 cm

Substrate Start Assay :

Pipette into a clean dry test tube labelled Test (T) :

Addition Sequence	**(T)** 25°C / 30°C / 37°C
Enzyme Reagent (L1)	0.8 ml
Sample	0.05 ml
Incubate at the assay temperature for 5 minutes and add	
Starter Reagent (L2)	0.2 ml

Mix well and read the initial absorbance A_0 after 5 minutes & repeat the absorbance reading after every 1, 2, & 3 minutes. Calculate the mean absorbance change per minute (ΔA / min.)

Sample Start Assay :

Pipette into a clean dry test tube labelled as Test (T) :

Addition Sequence	**(T)** 25°C / 30°C / 37°C
Working Reagent	1.0 ml
Incubate at the assay temperature for 1 minute and add	
Sample	0.05 ml

Mix well and read the initial absorbance A_0 after 10 minutes & repeat the absorbance reading after every 1, 2, & 3 minutes. Calculate the mean absorbance change per minute (ΔA / min.)

Calculations

Substrate / Sample start

CK - B activity in U/L 25°C / 30°C / 37°C = ΔA / min. x 3333

CK - MB activity in U/L 25°C / 30°C / 37°C = ΔA / min. x 6666

TEMPERATURE CONVERSION FACTORS

Assay Temperature	**Desired Reporting Temperature**		
	25°C	**30°C**	**37°C**
25°C	1.00	1.56	2.44
30°C	0.64	1.00	1.56
37°C	0.41	0.63	1.00

Linearity

This procedure is linear upto 1000 U/ml. Inhibition of CK - MM is upto 1500 U/L at 37°C. If the total CK activity exceeds this limit dilute the sample 1 + 9 with normal saline (NaCl 0.9%) before estimating CK - MB (Results x 10) .

Note

This method will also measure any CK - BB isoenzyme present in the sample. The amount of CK - BB is usually negligible in serum from normal individuals or in patients with myocardial infarction.

A macro form of BB has been observed and this will be measured as CK - B activity. If the CK - B activity exceedes 20% of the total CK activity the presence of macro BB should be suspected.

The working reagent or the combined reagent should have an absorbance below 0.800 against distilled water at 340 nm. Discard the reagent if the absorbance is above 0.800.

References

IFCC methods for the measurement of catalytic concentrations of enzymes, JIFCC. (1989) 1 : 130

System Parameters

Reaction	:	U.V. Kinetic
Wavelength	:	340 nm
Zero Setting	:	Distilled water
Incub. Temp.	:	37°C
Incub. Time	:	---
Delay Time	:	300 sec.
Read Time	:	180 sec.
No. of read.	:	4
Interval	:	60 sec.
Sample Vol.	:	0.05 ml
Reagent Vol.	:	1.00 ml
Standard	:	---
Factor	:	6666
React. Slope	:	Increasing
Linearity	:	1000 U/L
Units	:	U/L

CKB(Kin).02(P)

Coral Clinical Systems

Gitanjali, Dr. Antonio Do Rego Bagh,
Bambolim Complex P.O. GOA - 403 202, INDIA