

SBio CK MB (NAC act.) KIT

(Immunoinhibition / Modified IFCC Method)

(For invitro diagnostic use only)

REF	90342225	90332275
Pack Size	2 x 25 ml	2 x 75 ml



Store at 2-8°C	Manufacturer	In vitro Diagnostic Medical Device	Starter Reagent	This way up
Use by (Last day of stated month)	Consult Instructions for use	Batch Number	Immunoinhibition / Mod. IFCC	
Date of Manufacture	Catalogue Number	Enzyme Reagent	Immunoinhibition / Modified IFCC Method	Authorised Representative in the European Community

INTENDED USE

CK MB (NAC act.) Kit is used for the determination of CK-MB Activity in Serum.

PRINCIPLE OF THE TEST

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.

CLINICAL SIGNIFICANCE

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.

PRESENTATION

	2 x 25 ml	2 x 75 ml
L1 : Enzyme Reagent	2 x 20 ml	2 x 60 ml
L2 : Starter Reagent	2 x 5 ml	2 x 15 ml

COMPOSITION

Imidazole Buffer 100 mM; pH 6.6; Creatine Phosphate 30 mM; Glucose 20 mM; N-acetyl-L-cysteine 20 mM; Magnesium Acetate 10 mM; EDTA 2 mM; ADP 2 mM; NADP 2 mM; AMP 5 mM; DAPP 10 µM; G 6 PDH=2.0 KU/L; HK=2.5 KU/L, Anti CK-M antibodies inhibiting Human CK-M=1500 U/L, Non Reactive Stabilizers, Detergents and Preservatives.

STORAGE / STABILITY

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

SAMPLE REQUIRED

Serum. Free from haemolysis.

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 WASTE AND DISPOSAL

Do not reuse the reagent containers, bottles, caps or plugs due to the risks of contamination and the potential to compromise reagent performance.

This product requires the handling of human specimens. It is recommended that all human sourced material are considered potentially hazardous and are handled in accordance with the OSHA standard on blood borne pathogens.

Appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.

Handle specimens, solid and liquid waste and test components in accordance with local regulations and NCCLS guidelines M29, or other published biohazard safety guidelines.

PROCEDURE

Wavelength/filter	:	340 nm
Temperature	:	37°C / 30°C / 25°C
Light path	:	1 cm

MATERIALS REQUIRED BUT NOT PROVIDED

Photometer analyzer with standard thermostatic cuvette holder, micropipette and appropriate laboratory equipment.

Substrate Start Assay:

Pipette into a clean dry test tube labeled as 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 and repeat the absorbance reading after every 1, 2, & 3 minutes. Calculate the mean absorbance change per minute ($\Delta A/\text{min}$).

Sample Start Assay:

Pipette into a clean dry test tube labeled 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 and repeat the absorbance reading after every 1, 2, & 3 minutes. Calculate the

mean absorbance change per minute ($\Delta A/\text{min}$).

CALCULATIONS

Substrate / Sample start

CK - B activity in U/L $25^\circ\text{C} / 30^\circ\text{C} / 37^\circ\text{C} = \Delta A/\text{min} \times 3333$

CK - MB activity in U/L $25^\circ\text{C} / 30^\circ\text{C} / 37^\circ\text{C} = \Delta A/\text{min} \times 6666$

QUALITY CONTROL

The following process is recommended for QC during the assay of CK MB (NAC act.) Kit. *Define and establish acceptable range for your laboratory.

- Two levels of control (Normal and Abnormal) are to be run on a daily basis.
- If QC results fall outside acceptance criteria, recalibration may be necessary.
- Review QC results and run acceptance criteria following a change of reagent lot.

SPECIFIC PERFORMANCE CHARACTERISTICS

Linearity:

This procedure is linear upto 1000 U/L. 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).

Limit of detection:

The limit of detection for CK MB (NAC act.) Kit is 6 U/L.

Interferences:

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Precision:

Precision studies were performed with two controls using NCCLS protocol EP5-A. The results of the precision studies are shown below:

Sample	Within-run		Between-run		Total	
	Mean	CV%	Mean	CV%	Mean	CV%
Control 1	42.17	2.26	11.15	3.99	53.32	6.25
Control 2	75.47	3.42	32.81	5.68	108.28	9.1

Method comparison:

Comparative studies were done to compare our reagent with another

commercial CK MB (NAC act.) Assay. No significant differences were observed. Details of the comparative studies are available on request.

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

REFERENCE RANGE

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% to 25%

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

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 exceeds 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. The reagent may be used in several automated analyzers. Instructions are available on request. Do not use turbid, deteriorated or leaking reagents.

REFERENCES

- IFCC methods for the measurement of catalytic concentrations of enzymes, JIFCC. (1989) 1: 130.
- Clinical Chemistry, Principles, Procedures, Correlations, Michael L. Bishop et al., 5th Edition.



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