CK-MB

Creatine Kinase MB liquid reagent

CE

In Vitro Diagnostic reagent for the quantitative determination of Creatine Kinase (CK-MB) in serum and plasma. Store at 2-8°C .

REF: LCKMB-001 / LCKMB-002

Summary:

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 increased after an acute infarct of myocardium and later descends at normal levels. Also is increased, rarely, in skeletal muscle damage.

Principle:

Creatine Phosphate + ADP $\xrightarrow{CK}$ Creatine + ATP

ATP + Glucose $\xrightarrow{HK}$ ADP + Glucose-6-phosphate (G-6-P)

G-6-P + NADP+ $\xrightarrow{G6P\text{-}DH}$ 6-phosphogluconate + NADPH + H+

Composition:

R1: Imidazole Buffer pH 6.7 - 100 mmol/l, Glucose - 20 mmol/l, Magnesium Acetate - 10 mmol/l, EDTA - 2.0 mmol/l, ADP - 2.0 mmol/l, AMP - 5.0 mmol/l, NADP - 2.0 mmol/l, CK-M Inhibiting Antibody - 8000 U/l, HK - >2.5 U/ml, N-acetylcysteine - 20 mmol/l
R2: Creatine Phosphate - 30 mmol/l, G6P-DH - >1.5 U/ml, Diadenosine pentaphosphate - 10 umol/l, Preservatives
Calibrator: CKMB - Cal - lot specific

Precautions:

For In Vitro Diagnostics Use Only - For Professional Use Only
Carefully read instructions for use. Deviations from this procedure may alter performance of the assay.
Components Colour and Appearance:
Both Reagent: Clear colourless liquid.
Any significant changes could indicate that the assay might be compromised. Refer to Laboratory's QC program for actions to be taken. In case of serious damaged to the bottle and/or cap, resulting in product leakage and/or contamination: do not use the reagent pack and contact your distributor .
Safety precautions:
Contain small quantities of Sodium Azide. Material Safety Data Sheet is available upon request.

Instruments:

Instrument applications are available upon request.

Preparation:

Before use, mix reagent by gently inverting each bottle.
If stored and handled properly, unopened components are stable until the expiry date stated on the label
Monoreagent procedure: Mix 4 volumes of R1 with 1 volume of R2. Working reagent is stable 20 days at 2-8°C.
Bireagent procedure: Liquid reagent 1 and 2 are ready for use.

Samples:

Use serum free from haemolysis, heparin or EDTA plasma.
It is recommended to follow NCCLS procedures (or similar standardised conditions) regarding sample handling.
Serum/plasma should be separated from cells immediately after collection and stored in the dark. Stability: up to 7 days at 2-8°C.

Equipment:

- CK-MB Control: Photometer
- CK-MB Calibrator: General Laboratory Equipment

Procedure:

1. Assay conditions:
Wavelength: ..... λ: 340 (334/365) nm
Cuvette: ..... .1 cm. light path
Temperature ..... .30°C (25°C or 37°C)

2. Monoreagent procedure:

	Blank	Sample
R1 (µL)	1000	1000
Sample (µL)	-	40

Gently mix and Incubate at 30°C (25°C or 37°C) for 2 minutes, then measure the change of Optical Density per minute (ΔOD/min) for the next 3 minutes.

3. Bireagent procedure:

	Blank	Calibrator	Sample
R1 (µL)	800	800	800
Sample (µL)	-		40
Calibrator (µL)	-	40	-

4. Gently mix and Incubate at 37°C for 5 minutes

5. Add:

R2(µL)	200	200	200

6. Gently mix and Incubate at 37°C for 2 minutes, then measure the change of Optical Density per minute ΔOD/min) for the next 4 minutes.

7. Enzyme Calibration:
Using calibrator provided, calibrate the assay:
- When using a new reagent kit or changing lot number.
- Following preventive maintenance or replacement of a critical part of the photometer used.
- When Quality Controls are out of range.

Calculations:

$$\frac{\Delta OD\ Sample - \Delta OD\ Blank}{\Delta OD\ Calibrator - \Delta OD\ Blank} \times \text{Concentration of Calibrator.} = \text{CK MB Activity}$$

(Conversion factor: Qty in µKat/l = Qty in U/l x 0.0167).

Quality control:

All clinical laboratories should establish an Internal Quality Control program. Check instrument and reagent performance with recommended controls or similar. The values obtained for QC should fall within manufacturer's acceptable ranges or should be established according to the Laboratory's QC program.
Controls should be assayed:
- Prior reporting patient results.
- Following any maintenance procedure on the photometer used.
- At pre-established intervals following the Q.C. Laboratory recommandations.

Reference values:

The probability of myocardial injury is high when the following 3 conditions are met:

			U/L at 25°C	µkat/l at 25°C	U/L at 30°C	µkat/l at 30°C	U/L at 37°C	µkat/l at 37°C
1.	CK	Men	> 80	> 1.33	> 130	> 2.17	>190	> 3.17
		Women	> 70	> 1.17	> 110	> 1.83	> 167	> 2.87
2.	CK- MB		> 10	> 0.17	> 15	> 0.25	> 24	> 0.40
3.	CK-MB activity accounts for 6 – 25% of the total CK activity							

* Calculated values. Temperature conversion factors: 25°C to 30°C: 1.53. 25°C to 37°C: 2.38

Performance characteristics:

Performance results can vary with the instrument used. Data obtained in each individual laboratory may differ from these values.
Linearity:
Linear up to 318 U/L.
For samples with a higher concentration, dilute 1:1 with 0.9% NaCl (9g/l) and re-assay. Multiply result by 2.
Interfering substances:
Bilirubin (mixed isomers): Less than 10% interference up to 600µmol/l Bilirubin.
Haemolysis: Less than 10% interference up to 1.25 g/l Haemoglobin.
Lipemia: Less than 10% interference up to 2.5 g/l Intralipid.
Sensitivity:
The Lowest Detectable Level is estimated at 2 U/l (0.03 µkat/l).
Precision:

	Intra-assay			Inter-assay		
N=20	Mean (U/L)	SD	%CV	Mean (U/L)	SD	%CV
level 1	172.1	4.88	2.83	165.4	5.58	3.37
level 2	776.4	13.46	1.73	740.2	15.26	2.06

Method comparison:
Using 50 samples, a comparison, between this CK MB test (y) and another commercially available test (x), gave the following results:
y = 0.976x – 0.269
r = 0.999
Sample range: 0 to 329 U/l

References:

1. Stein W. Med Weit. 1985, 36:572 & Burtis CA, Ashwood ER. Tietz Fund. Of Clin. Chem. 5th ed. 30-54, 352-390 and 974-975
2. GuderWG, NarayananS, WisserH, ZawtaB. List of Anal; Preanal Variables. From the Patient to the Laboratory. Darmstdt:GIT Verlag 1996.
3. Wurzburg U, Hennrich N, Lang H, Prellwitz W, Neumeier D, Knedel M. Klin. Wschr. 1976: 54 and 357.
4. Szasz G, Busch EW. Third European Congress of Clinical Chemistry, Brighton, England, 3-8 June 1979 (abstract).

Sorachim