CE

CK - MB - LO

CK-MB-LQ (Creatine kinase – MB)

Anti CK-M. Immunoinhibition. Kinetic UV. Liquid

Quantitative determination of creatine kinase MB (CK-MB) IVD

Store at 2-8ºC

PRINCIPLE OF THE METHOD

The procedure involves measurement of CK activity in the presence of an antibody to CK-M monomer. This antibody completely inhibits the activity of CK-MM and half of the activity of CK-MB while not affecting the B subunit activity of CK-MB and CK-BB. Then it's used the CK method to quantitatively determine CK-B activity[1,2]. The CK-MB activity is obtained by multiplying the CK-B activity by two.

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 increased after an acute infarct of myocardium and later descends at normal levels. Also is increased, rarely, in skeletal muscle damage[5,6,7,8].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R 1	Imidazol, pH 6.7	125 mmol/L
	D-Glucose	25 mmol/L
	N-Acetyl-L-Cysteine	25 mmol/L
	Magnesium acetate	12,5 mmol/L
	NADP	2,52 mmol/L
	EDTA	2,02 mmol/L
	Hexokinase	≥6 800 U/L
Anti-human polyclonal CK-M antibody (sheep) sufficient to inhibit up to 2000 U/L of CK-MM		
R 2	ADP	15,2 mmol/L
	AMP	25 mmol/L
	di-Adenosine-5- pentaphosphate	103 mmol/L
	Glucose-6-phosphate dehydrogenase	≥8 800 U/L
	Creatine phosphate	250 mmol/L

Optional

CK-NAC / CK-MB CONTROL	Lyophilized human serum

PREPARATION

Working reagent (WR): Mix 4 volumes of reagent 1 with 1 volume of reagent 2.
Stability: 7 days at 2-8ºC or 12 hours at room temperature (20-25ºC).

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC, protected from light and contaminations prevented. Do not use reagents over the expiration date.
Signs of reagent deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 340 nm ≥ 1,2.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 340 nm.
- Thermostatic bath at 25ºC, 30ºC ó 37º C (± 0,1ºC).
- Matched cuvettes 1,0 cm light path.
- General laboratory equipment.

SAMPLES

Serum free of hemolysis or heparin plasma: Stability 15 days at -20ºC, protected from light.
CK-MB activity decreases a 10% after 24 hours at 4ºC or 1 hour at 25ºC.

PROCEDURE

1. Assay conditions:
   Wavelength: . . . . . 340 nm
   Cuvette: . . . . . 1 cm light path
   Constant temperature . . . . . 25ºC / 30ºC / 37ºC
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 and incubate 10 minutes.
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 of CK-B ΔA x 1651 = U/L of CK-MB

Calculating factor in automatic analyzers by kinetic method (ΔA/min.) is 8255.
Units: One international unit (IU) is the amount of enzyme that transforms 1µmol of substrate per minute, in standard conditions. The concentration is expressed in units per litre of sample (U/L).

Temperature conversion factors

To correct results to other temperatures multiply by:

Assay temperature	Conversion factor to		
	25ºC	30ºC	37ºC
25ºC	1,00	1,53	2,38
30ºC	0,65	1,00	1,56
37ºC	0,42	0,64	1,00

QUALITY CONTROL

CK-NAC/CK-MB specific control sera is 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

The suspicion of myocardial damage is based on the three following factors:

CK-MB	25ºC	30ºC	37ºC
	< 10 U/L	< 15 U/L	< 24 U/L
TOTAL CK	25ºC	30ºC	37ºC
Men, up to	80 U/L	130 U/L	195 U/L
Women, up to	70 U/L	110 U/L	170 U/L

$$\frac{\text{CK - MB Activity}}{\text{CK Total Activity}} \times 100 = 6 - 25\ \%\ \text{CK Total}$$

These values are for orientation purpose. Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From *detection limit* of 1,9 U/L to *linearity limit* of 318 U/L.
If the results obtained were greater than linearity limit, dilute the sample 1/1 with NaCl 9 g/L and multiply the result by 2.
Precision:

	Intra-assay		Inter-assay	
Mean (U/L)	33,7	166,5	31,3	161,0
SD	1,00	3,76	1,19	3,47
CV (%)	2,96	2,26	3,81	2,15

Sensitivity: 1 U/L = 0,000134 (A).
Accuracy: Results obtained using Audit Diagnostics reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained were the following:
Correlation coefficient $(r)^2$: 0,999.
Regression equation: y= 0,976 x + 0,269.
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Bilirubin (mixed isomers): Less than 10% interference up to 600 µmol/L Bilirubin.
Hemolysis: Less than 10% interference up to 1,25 g/L Hemoglobin.
Lipemia: Les than 10% interference up to 2,5 g/L Intralipid.
A list of drugs and other interfering substances with CK determination has been reported[3,4].

LIMITATION OF THE PROCEDURE

The method will also measure any CK-BB isoenzyme present in serum. The activity of the isoenzyme is usually negligible, however, if a significant amount of CK-BB activity is present the CK-MB activity will be overestimated.
A macro form of BB (immunoglobulin complexed) has been observed wich will be measured as B in the assay. If the measured CK-B activity exceeds 20% of the total CK activity, the presence of macro BB should be suspected.

NOTES

Audit Diagnostics has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

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