CK-MB-LQ (Creatine kinase – MB)

Anti CK-M. Immunoinhibition. Kinetic UV. Liquid

Quantitative determination of creatine kinase (CK-MB)

CE IVD

PACKAGING

Ref.: 101-0646	Cont.: 1 x 200 / 1 x 50 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 increased 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

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.

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 2 000 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 (not included in the kit)

CK-NAC / CK-MB CONTROL			
Level 1	Ref.: 101-0697	1 x 2 mL	Lyophilized human control serum
Level 2	Ref.: 101-0762	4 x 5 mL	Lyophilized human control serum

PREPARATION

Mix 4 volumes of reagent 1 with 1 volume of reagent 2.

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 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, 30° C or 37º C (± 0.1º C).
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

Serum free of hemolysis or heparin 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 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 (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).

Temperature conversion factors

To correct results to other temperatures multiply by:

Assay temperature	Conversion factor to		
	25º C	30º C	37º C
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

Control sera are 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	25º C	30º C	37º C
	> 10 U/L	> 15 U/L	> 24 U/L

TOTAL CK	25º C	30º C	37º C
Men, up to	80 U/L	130 U/L	195 U/L
Women, up to	70 U/L	110 U/L	170 U/L

$\frac{\text{CK} - \text{MB Activity}}{\text{CK Total Activity}} \times 100$: 6 - 25 % CK - MB Activity in the sample

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

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PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit of 1 U/L (on Cobas Mira) to linearity limit of 600 U/L (on manual method and on Cobas Mira).

If the results obtained were greater than linearity limit, dilute the sample 1/10 with NaCl (9 g/L) and multiply the result by 10.

Precision:

	Intra-assay		Inter-assay	
Mean (U/L)	24.95	66.0	25.0	74.0
CV (%)	10.36	4.59	9.80	2.62

Sensitivity: 10 U/L (on Cobas Mira).

Accuracy: Results obtained using CHRONOLAB reagents (y) did not show systematic differences when compared with other commercial reagents (x).

The results obtained were the following:

Correlation coefficient (r): 0.99.

Regression equation: y= 1.0183 x + 0.308.

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

No interferences were observed with glucose until 7 g/L, hemoglobin until 6 g/L and triglycerides 8 mmol/L. A list of drugs and other interfering substances with CK determination has been reported by Young et. al[3,4].

LIMITATION OF THE PROCEDURE

1- 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.

2- 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.

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.
7. Mathieu M. et coll. Recommandation pour la mesure de la concentration catalytique de la créatinine kinase dans la sérum humain. Ann. Biol. Clin.,40, (1482), 87.
8. Neumeier, D., Prellwitz, W., Würzburg, U. et coll. Determination of creatine kinase isoenzyme MB activity in serum using immunological inhibition of creatine kinase M subunit acitivity. Activity kinetics and diagnostic significance in myocardial infarcton, Clin. Chim. Acta, 73, (1976), 445.

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