CREATINE KINASE-MB (CK-MB)

BioSystems

COD 12566 3 x 15 mL
Only for *in vitro* use in the clinical laboratory

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CREATINE KINASE-MB (CK-MB)
Immunoinhibition

INTENDED USE

Reagent for the measurement of creatine kinase-MB (CK-MB) concentration in human serum or plasma. The obtained values are useful as an aid in diagnosis and control of the evolution of acute myocardial infarction.

This reagent is for use in the BioSystems A25 and A15 analyzers or in other analyzer with similar performance characteristics.

CLINICAL SIGNIFICANCE

Creatine kinase is composed of two polypeptide chains, denoted B (for brain) and M (for muscle); these give the three dimeric isoenzymes: MM (CK-1), MB (CK-2) and BB (CK-3).

The percentages of CK-MB activity versus total CK activity are usually less than 6 %, but after a myocardial infarction, these values can rise from 10 to 30% depending on the extent of myocardial damage and the location of the infarct. However, a myocardial infarction in a previously healthy heart may have a rather low serum CK-MB fraction. Therefore, the diagnosis of myocardial damage must be based on the clinical history and findings, the magnitude of the CK-MB elevation, and its temporal pattern[1,2].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

PRINCIPLE OF THE METHOD

A specific antibody inhibits both M subunits of CK-MM (CK-3), and the single M subunit of CK-MB (CK-2) and thus allow determination of the B subunit of CK-MB (assuming the absence of CK-BB or CK-1)[3,4]. CK-B catalytic concentration, which corresponds to half of CK-MB concentration, is determined from the rate of NADPH formation, measured at 340 nm, by means of the hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6P-DH) coupled reactions[5].

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow{\text{CK-B}} \text{Creatine} + \text{ATP}$$

$$\text{ATP} + \text{Glucose} \xrightarrow{\text{HK}} \text{ADP} + \text{Glucose - 6 - phosphate}$$

$$\text{Glucose - 6 - phosphate} + \text{NADP}^+ \xrightarrow{\text{G6P-DH}} \text{Gluconate - 6 - phosphate} + \text{NADPH} + \text{H}^+$$

CONTENTS AND COMPOSITION

A. Reagent: 3 x 12 mL. Anti-human-CK-M able to inhibit 2000 U/L of CK-M, Imidazol 125 mmol/L, EDTA 2 mmol/L, magnesium acetate 12.5 mmol/L, D-glucose 25 mmol/L, N-acetyl cysteine 25 mmol/L, hexokinase 6800 U/L, NADP 2.4 mmol/L, pH 6.1.

DANGER: H360: May damage fertility or the unborn child. P201: Obtain special instructions before use. P202: Do not handle until all safety precautions have been read and understood. P280: Wear protective gloves/protective clothing/eye protection/face protection. P308+P313: IF exposed or concerned: Get medical advice/attention. P405: Store locked up.

B. Reagent: 1 x 10 mL. Creatine phosphate 250 mmol/L, ADP 15.2 mmol/L, AMP 25 mmol/L, P1, P5-di(adenosine-5'-)pentaphosphate, 103 µmol/L, glucose-6-phosphate dehydrogenase 8800 U/L.

For further warnings and precautions, see the product safety data sheet (SDS).

STORAGE AND STABILITY

Store at 2-8ºC.

Components are stable once opened until the expiry date marked in the label if they are stored well closed and care is taken to prevent contamination during their use.

On board stability: Reagents open and kept in the refrigerated compartment of the analyzer are stable 15 days.

Indications of deterioration: Absorbance of the blank over the limit indicated in "Test Parameters".

ADDITIONAL MATERIALS REQUIRED (NOT PROVIDED)

S. Creatine Kinase-MB (CK-MB) Standard 1 x 1 mL (BioSystems Cod. 11824). Human CK-MB. CK-MB concentration is given on the vial label. CK-MB value is traceable to the reference material ERM-AD455/IFCC (IRMM).

Components from human origin have been tested and found to be negative for the presence of antibodies anti-HIV and anti-HCV, as well as for Hbs antigen. However, they should be handled cautiously as potentially infectious.

Reconstitute with 1.0 mL of distilled water. Stable for 7 days at 2-8ºC or 2 month at –20ºC (only freeze once).

REAGENT PREPARATION

Working Reagent: Add 3.0 mL of the Reagent B into the Reagent A bottle. Mix gently. Other volumes can be prepared in the proportion: 4 mL Reagent A + 1 mL Reagent B.

Stable for 15 days at 2-8ºC. The working reagent must be protected from light.

SAMPLES

Serum or heparinized plasma collected by standard procedures.

Total CK concentration in the sample must be lower than 1000 U/L. Dilute the serum 1/2 if necessary, with NaCl 150 mmol/L.

CK-MB is stable for 7 days at 2-8ºC.

CALIBRATION

A reagent blank should be done every day and a calibration at least every 15 days, after reagent lot change or as required by quality control procedures.

QUALITY CONTROL

It is recommended to use the CK-MB Control Serum (cod. 18024 and cod. 18061) to verify the accuracy of the measurement procedure. CK and CK-MB concentrations are given on the vial label. CK value is traceable to the reference system as described by the IFCC Committee on Reference Systems for Enzymes and CK-MB value is traceable to the reference material ERM-AD455/IFCC (IRMM). Traceability can be assured only if the BioSystems reagents and recommended measurement procedures are used.

Components from human origin have been tested and found to be negative for the presence of antibodies anti-HIV and anti-HCV, as well as for Hbs antigen. However, they should be handled cautiously as potentially infectious.

Reconstitute the serum with the volume of distilled water indicated in the label. Stable for 7 days at 2-8ºC or 2 month at –20ºC (only freeze once). Treat the Control in the analytical procedure as patient samples.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

REFERENCE VALUES

The discrimination value for myocardial infarction is around 25 U/L = 0.42 µkat/L. However, an index higher than 6% of total CK concentration[1] discriminates better.

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

METROLOGICAL CHARACTERISTICS

The metrological characteristics described below have been obtained using an A25 analyzer. Results are similar with A15.

- Detection limit: 1.15 U/L = 0.019 µkat/L.
- Linearity limit: 1000 U/L = 16.7 µkat/L. For higher values dilute sample 1/2 with distilled water and repeat measurement.
- Precision:

Mean concentration	Repeatability (CV)	Within-laboratory (CV)
39.1 U/L = 0.74 µkat/L	1.1 %	1.9 %
92.8 U/L = 1.47 µkat/L	0.5 %	1.1 %

- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

LIMITATIONS OF THE PROCEDURE

- Interferences: bilirubin (up to 20 mg/dL), hemolysis (hemoglobin up to 250 mg/dL) and lipemia (triglycerides up to 125 mg/dL) do not interfere. Presence in the sample of above normal concentrations of CK-BB or adenilate kinase, and of macro or mitochondrial CK interfere[6]. Other drugs and substances may interfere[7].

BIBLIOGRAPHY

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3. Würzburg U, Hennrich N, Lang H, Prellwitz W, Neumeier D and Knedel M. Bestimmung der aktivität von creatinkinase MB im serum unter verwendurng inhibierender antikörper. *Klinische Wochenschrift* 1976; 54: 357-360.
4. Gerhardt W and Waldenstrom G. Creatine kinase B-subunit activity in serum after immunoinhibition of M-subunit activity. *Clin Chem* 1979; 25: 1274-1279.
5. IFCC methods for the measurement of catalytic concentration of enzymes. Part 7: IFCC method for creatine kinase. *JIFCC* 1989; 1: 130-139.
6. Urdal P and Landaas S. Macro creatine kinase BB in serum, and some data on its prevalence. Clin Chem 1979; 25: 461-465.
7. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.

TEST PARAMETERS

These reagents may be used in several automatic analyzers. Specific instructions for application in many of them are available on request.

R1: use Reagent A, R2: use Reagent B.

	A25	A15
GENERAL		
Name	**CK-MB**	**CK-MB**
Sample type	SER	SER
Analysis mode	fixed-time mon.	fixed-time mon.
Units	U/L	U/L
Turbidimetry test	no	no
Decimals	0	0
Type of reaction	increasing	increasing
PROCEDURE		
Reading mode	monoch.	monoch.
Main filter	340	340
Reference filter	-	-
Sample	12	12
Vol. R1	300	300
Vol. R2	-	-
Washing	1.2	1.2
Reading 1 (cycle)	21	14
Reading 2 (cycle)	41	26
Reagent 2 (cycle)	-	-
Predilution factor	-	-
CALIBRATION AND BLANK		
Calibration type	specific	specific
Number of calibrators	-	-
Calibration curve	-	-
OPTIONS		
Blank absorbance limit	0.400	0.400
Kinetic blank limit	-	-
Linearity limit	1000	1000
Substrate depletion	-	-

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