COD 11792 1 x 50 mL
STORE AT 2-8ºC
Reagents for measurement of CK-MB concentration Only for *in vitro* use in the clinical laboratory

CREATINE KINASE-MB (CK-MB)

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BioSystems

CREATINE KINASE-MB (CK-MB)
Immunoinhibition

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)[1,2]. 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[3].

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

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

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

COMPOSITION

A. Reagent. 1 x 40 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

Store at 2-8ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagents: Presence of particulate material, turbidity, absorbance of the blank over 0.400 at 340 nm (1 cm cuvette).

AUXILIARY REAGENTS

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: Pour the contents 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.

ADDITIONAL EQUIPMENT

– Analyzer, spectrophotometer or photometer with cell holder thermostatable at 37ºC and able to read at 340 nm.

– Cuvettes with 1 cm light path.

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.

PROCEDURE

1. Bring the Working Reagent and the instrument to 37ºC.
2. Pipette into labelled test tubes: (Note 1)

Sample	40 µL
Working Reagent	1.0 mL

3. Mix thoroughly and incubate immediately at 37ºC. Start the stopwatch.
4. Read the absorbance (A) at 340 nm after exactly 5 minutes (A_5) and 10 minutes (A_{10}) of incubation.

CALCULATIONS

The CK-MB concentration in the sample is calculated using the following formula:

$$(A_{10} - A_5) \times \frac{Vt \times 10^6}{\varepsilon \times l \times Vs \times 5\ min} \times 2 = U/L$$

The molar absorbance (ε) of NADPH at 340 nm is 6300, the lightpath (l) is 1 cm, the total reaction volume (Vt) is 1.04, the sample volume (Vs) is 0.04, and 1 U/L are 0.0167 µkat/L. The following formulas are deduced for the calculation of the catalytic concentration:

$A_{10} - A_5$	x 1651 = U/L x 27.5 = µkat/L

If the Creatine Kinase-MB (CK-MB) Standard is used to calibrate:

$$\frac{(A_{10} - A_5)_{Sample}}{(A_{10} - A_5)_{Standard}} \times C_{Standard} = C_{Sample}$$

The CK-MB index is calculated using the following formula:

$$\frac{CK_{MB}\ concentration}{CK_{total}\ concentration} \times 100 = \%$$

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[4] discriminates better.

QUALITY CONTROL

It is recommended to use the CK-MB Control Serum (cod. 18024 and cod. 18061) to verify the performance 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.

The intervals of suggested acceptable values have been calculated from previous experience in interlaboratory variability and are given for orientation only; each laboratory should establish its own precision parameters.

METROLOGICAL CHARACTERISTICS

– Detection limit: 3 U/L = 0.05 µkat/L.

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

– Repeatibility (within run):

Mean Concentration	CV	n
45 U/L = 0.75 µkat/L	2.8 %	20
129 U/L = 2.15 µkat/L	2.3 %	20

– Reproducibility (run to run):

Mean Concentration	CV	n
45 U/L = 0.75 µkat/L	3.5 %	25
129 U/L = 2.15 µkat/L	3.2 %	25

– 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.

– Interferences: Hemolysis (hemoglobin > 2.5 g/L) and lipemia (triglycerides > 1.25 g/L) interfere. Presence in the sample of above normal concentrations of CK-BB or adenilate kinase, and of macro or mitochondrial CK interfere[5]. Bilirubin (< 20 mg/dL) does not interfere. Other drugs and substances may interfere[6].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

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[4,7].

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

NOTE

1. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.

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