CREATINE KINASE - MB CE

REF 1121005
1 x 25 mL
CONTENTS
R1. Reagent 1 x 20 mL
R2. Reagent 1 x 5 mL

For *in vitro* diagnostic use only

CREATINE KINASE - MB
CK –MB INHIBITED
Immunological UV method
KINETIC

PRINCIPLE

The major CK activity in normal serum is due to CK-MM and CK-MB isoenzymes, found mainly in skeletal and heart muscle. CK-BB is ussualy present in serum at low concentration.
Both, creatine kinase (CK) enzymes are dimers formed by the association of two sub-units from muscle (M) and nerve cells (B). The immunoinhibition from an specific antibody of both, MM sub-units and the single M sub-unit of CK-MB, allows the determination of the B sub-unit. The CK-B activity corresponding to half of CK-MB is measured by the increasing rate of absorbance resulting from the following coupled reactions.[1,2]

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow[\text{pH 6.5}]{\text{CK (AMP, NAC)}} \text{Creatine} + \text{ATP}$$

$$\text{ATP} + \text{Glucose} \xrightarrow{\text{HK}} \text{ADP} + \text{G6P}$$

$$\text{G6P} + \text{NADP}^+ + \text{H}_2\text{O} \xrightarrow{\text{G6P-DH}} \text{Gluconate-6P} + \text{NADPH} + \text{H}^+$$

This test has been formulated according the standarized method described by IFCC. Clin Chem Lab Med 2002; 40(6) : 635-642.

REAGENT COMPOSITION

R1 **Buffer/Glucose/NAC.** Imidazol buffer 100 mmol/L pH 6.7, glucose 20 mmol/L, NAC 20 mmol/L, magnesium acetate 10 mmol/L, NADP 2.5 mmol/L, HK ≥ 4 KU/L, EDTA 2 mmol/L.

R2 **Substrate/Coenzymes.** CP 30 mmol/L, AMP 5 mmol/L, ADP 2 mmol/L, di(adenosine-5´) pentaphosphate 10 µmol/L, G6P-DH ≥ 1.5 KU/L.
Sufficient CK-M human antibody to inhibit ≥ 3000 U/L of CK-MM at 37ºC.

STORAGE AND STABILITY

Store at 2-8ºC.
All the kit compounds are stable until the expiry date stated on the label. Do not use reagents over the expiration date.
Store the vials tightly closed, protected from light and prevented contaminations during the use.
Discard If appear signs of deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 340 nm > 0.400 in 1cm cuvette.

REAGENT PREPARATION

Working reagent. Mix 4 mL of **R1** + 1 mL of **R2**. Stable for 1 month at 2-8ºC or 5 days at 20-25ºC.
Protect from light.

SAMPLES

Serum. Stable 8 days at 2-8ºC or 1 month at –20ºC. Chill the samples as rapidily as possible after collection.
Moderately or severely hemolyzed specimens are unsatisfactory for testing, as well as plasmas containing EDTA, heparin, citrate, or fluoride since they may produce unpredictable reaction rates.

INTERFERENCES

- Lipids (intralipid >1 g/L) may affect the results.
- Bilirubin (< 20 mg/dL) does not interfere.
- Hemoglobin (>8 g/L) may affect the results.
- The presence of above normal concentrations of CK-BB or adenilatekinasa, and of macro or mitochondrial CK may affect the results [6].
- Other drugs and substances may interfere[8-9].

MATERIALS REQUIRED

- Photometer or spectrophotometer with a thermostatted cell compartment set at 25/30/37ºC, capable of reading at 340 nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm pathlength.
- Pipettes to measure reagent and samples.

PROCEDURE

A. Determination of total CK activity

Test the total activity of creatinine kinase at 37ºC using Linear CK-NAC reagents, Ref. 1120005 or 1120010.
Samples with activities higher than 2000 U/L should be diluted 1:10 with saline and assayed again. Multiply the results by 10.

B. Determination of CK-B activity

Sample starter

Test the activity of CK-B at 37ºC.

1. Set the photometer to 0 absorbance with distilled water.
2. Pipette into a cuvette:

Working reagent	1.0 mL
Sample or control	40 µL

3. Mix gently by inversion. Insert cuvette into the cell holder and start stopwatch.
4. Incubate for 5 minutes and record initial absorbance reading.
5. Repeat the absorbance readings exactly after 1, 2 and 3 minutes. Calculate the difference between absorbances and the mean of
6. the results to obtain the average change in absorbance per minute (ΔA/min).

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Substrate starter

Test the activity of CK-B at 37ºC.

1. Set the photometer to 0 absorbance with distilled water.
2. Pipette into a cuvette:

R2	250 µL
Sample or control	50 µL

3. Mix. Incubate for 2 minutes and add:

R1	1.0 mL

4. Incubate for 5 minutes and record initial absorbance reading.
5. Repeat the absorbance readings exactly after 1, 2 and 3 minutes.
6. Calculate the difference between absorbances and the mean of the results to obtain the average change in absorbance per minute (ΔA/min).

CALCULATIONS

Total CK activity U/L = ΔA x 8095 (37ºC)
CK-B activity U/L = ΔA x 4180

Multiply CK-B activity by 2 to obtain activity of CK-MB (U/L).

Calculations are common to both procedures.

Percentaje of CK-MB activity

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

REFERENCE VALUES

The normal range of CK-MB activity in the serum of adults is considered to be 2.0 U/L to 19.5 U/L at 37ºC. Newborns, infants, and children have higher serum CK-MB values than adults.
A ratio between CK-MB and total CK activities above 4% should be considered suspicious, and above 10% consistent with acute myocardial infarction.[4]
When the three conditions stated below are met the probability of myocardial injury is very high.[5]

Condition	Test temperature (37º)	
1	CK Men	> 190 U/L (3.17 µkat/L)
	CK Women	> 167 U/L (2.78 µkat/L)
2	CK-MB	> 24 U/L (0.40 µkat/L)
3	% CK-MB	6-25%

It is recommended that each laboratory establishes its own reference range.

QUALITY CONTROL

To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

CLINICAL SIGNIFICANCE

The major medical application of CK-MB assays is in adults for the diagnosis of acute myocardial infarction (AMI) or, the differentiation of myocardial injury from skeletal muscle damage. Creatine kinase-MB is considered one of the best laboratory indicators of AMI. CK-MB determination within the proper time frame after infarction is most critical, the useful interval ("window") being from about 10 to 24 hour after the infarction. Its detection is of importance in determining the degree of the injury and the efficacy of the treatment.

ANALYTICAL PERFORMANCE

- ***Detection Limit :*** 6.66 U/L
- ***Linearity :*** Up to 330 U/L
- ***Precision:***

U/L	Within-run		Between-run	
Mean	36.7	195.5	36.7	195.5
SD	1.92	5.76	5.27	3.76
CV%	5.27	2.94	3.15	1.92
N	10	10	10	10

- ***Sensitivity :*** 0.120 mA / min/ U/L CK-MB.
- ***Correlation:*** This assay (y) was compared with a similar commercial method (x). The results were:
  N = 44 r = 0.99 y = 0.938x + 1.345

The analytical performances have been generated using on automatic instrument. Results may vary depending on the instrument.

NOTES

1. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meets the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact to the distributor for any question on the application method.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

REFERENCES

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B1121-2/0901
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QUALITY SYSTEM CERTIFIED
ISO 9001 ISO 13485

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