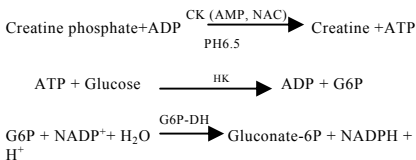


## CREATINE KINASE – MB CK-MB INHIBITED Immunological UV method KINETIC

### 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 usually 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.



### 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  $\geq 4$  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  $\mu$ mol/L, G6P-DH  $\geq 21.5$  KU/L.

Sufficient CK-M human antibody to inhibit  $\geq 3000$  U/L of CK-MM at 37°C.

### STORAGE AND STABILITY

Store at 2-8°C.

The Reagents are stable until the expiry date stated on the label.

### REAGENT PREPARATION

Working reagent. Mix 4 mL of R1 + 1 mL of R2. Stable for 1 month at 2-8°C or 10 days at 16-25°C. Protect from light. Discard the reagent if presents an absorbance over 0.300 at 340 nm against distilled water or if it fails to recover the declared values of control sera.

### SAMPLES

Serum. Stable 8 days at 2-8°C or 1 month at -20°C. Chill the samples as rapidly 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

– A number of drugs has been listed that will affect the CK determination.<sup>3</sup>

– The method also measures any CK-BB present in serum. Its activity is usually negligible.

### MATERIALS REQUIRED BUT NOT PROVIDED

- Photometer or spectrophotometer with a thermostated cell compartment set at 25/30/37°C, capable to read 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 37°C using Atlas CKNAC reagents.

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 37°C.

1- Set the photometer to 0 absorbance with distilled water.

2- Pipette into a cuvette:

Working reagent	1.0 mL
Sample or control	40 $\mu$ L

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.

6- Calculate the difference between absorbances and the mean of the results to obtain the average change in absorbance per minute ( $\Delta A/\text{min}$ ).

##### Substrate starter

Test the activity of CK-B at 37°C.

1- Set the photometer to 0 absorbance with distilled water.

2- Pipette into a cuvette:

<b>R2</b>	<b>250 <math>\mu</math>L</b>
<b>Sample or control</b>	<b>50 <math>\mu</math>L</b>

3- Mix. Incubate for 2 minutes and add:

<b>R1</b>	<b>1.0 mL</b>
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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 ( $\Delta A/\text{min}$ ).

### CALCULATIONS

Total CK activity U/L =  $\Delta A \times 8095$  (37°C)

CK-B activity U/L =  $\Delta A \times 4180$

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

Calculations are common to both procedures.

Percentage of CK-MB activity

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

Total CK 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 37°C. 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.<sup>1</sup>

When the three conditions stated below are met the probability of myocardial injury is very high.

Condition	Test temperature (37°)	
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.

#### 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 being the useful interval ("window") 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

- *Linearity.* Up to 330 U/L

- *Precision*

U/L	Within-run			Between-run		
Mean	16.3	56.3	96.7	16.1	53.5	98.5
SD	0.32	0.64	1.072	0.33	1.46	1.37
CV%	1.96	1.14	1.1	2.04	2.73	1.4
N	10	10	10			

Replicates: 10 for each level. Replicates 10 for each level

Instrument: COBAS MIRA for 6 days.

- *Sensitivity.* Using this reagent and method an AA/min of 0.001 read at 340 nm is equivalent to 4.1 U/L of CK-MB activity.

- *Correlation.* This assay (y) was compared with a similar commercial method (x). The results were:

N=25      r=0.997      y=0.958 x-0.55

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