



# CK-MB NAC

unitest

For CK-MB determination in serum or plasma

## MONOCLONAL

### SUMMARY

Creatine Kinase (CK) is an intramuscular enzyme constituted by a subunit M (muscle) and other subunit B (brain) which combine giving place to isoenzymes CK-MM (muscular), CK-BB (brain) and CK-MB (myocardial).

Serum increase of CK and of CK-MB is an indicator of myocardial injury. After an acute myocardial infarction, in approximately 55% of the cases, the highest peak increase of CK and CK-MB is simultaneously produced, while in 45% of the cases the highest increase of CK-MB precedes the total CK.

### PRINCIPLE

The method is based on the specific inhibition of the CK-M subunits with monoclonal antibodies anti CK-M. The antibodies inhibit not only the MM isoenzyme but also the M subunits corresponding to CK-MB. Subunits B are determined through the use of a reactive system based on an analytical technique optimized by the IFCC, with N-acetyl-cysteine as activator, adding monoclonal antibodies anti-CK-M.

### PROVIDED REAGENTS

**A. Reagent A:** single assay vials containing enough quantities to obtain the following concentrations once reconstituted:

Creatine phosphate .....	30 mmol/l
ADP .....	2 mmol/l
Glucose .....	20 mmol/l
NADP.....	2 mmol/l
Hexokinase (HK) .....	≥ 2 500 U/l
Glucose-6-phosphate dehydrogenase .....	≥ 2 000 U/l
Magnesium acetate .....	10 mmol/l
AMP.....	5 mmol/l
Di (adenosine-5') pentaphosphate .....	10 umol/l
N-acetyl-cysteine (NAC).....	20 mmol/l
Monoclonal antibodies capable of inhibiting 1000 U/l of CK-M.	

**B. Reagent B:** 100 mmol/l imidazole buffer solution, pH 6.7.  
**Control:** vial containing lyophilized human CK-MB (see attached table for theoretic value).

### INSTRUCTIONS FOR USE

**Reagent B:** ready to use.

**Reagent A:** add 2.5 ml Reagent B to a Reagent A vial. Cap and shake until complete dissolution.

**Control:** open the vial carefully trying not to spill the content. Measure with pipette exactly 1.0 ml of distilled water. Cap and wait for 5 minutes. Dissolve the content of the vial completely by inversion. The reconstituted CK-MB Control is treated in the same way as an unknown sample.

### WARNINGS

Reagents are for "in vitro" diagnostic use.

The Control has been tested for HIV, HCV and HBV being found non-reactive. Nonetheless, it should be handled as infectious material.

Use the reagents according to the working procedures for clinical laboratories.

The reagents and samples should be discarded according to the local regulations in force.

### STABILITY AND STORAGE INSTRUCTIONS

**Provided Reagents:** stable in refrigerator (2-10°C) until the expiration date shown on the box.

**Reconstituted Reagent A:** stable in refrigerator (2-10°C) for 3 days after reconstitution date.

**Reconstituted Control:** stable in refrigerator (2-10°C) for 3 days or 3 months in freezer (-20°C). Do not freeze and thaw repeatedly.

### INSTABILITY OR DETERIORATION OF REAGENTS

When spectrophotometer has been set to zero with distilled water, absorbance readings of reconstituted Reagent A higher than 0.800 O.D. (at 340 nm) indicate deterioration.

### SAMPLE

Serum or plasma

**a) Collection:** obtain in the usual way.

**b) Additives:** when using plasma, heparin or EDTA must be used as anticoagulant. The use of Wiener lab.'s **Anticoagulante W** is recommended.

**c) Known interfering substances:** sera with visible or intense hemolysis should not be used as they produce falsely increased values.

See Young, D.S. in References for effect of drugs on the present method.

**d) Stability and storage instructions:** sample should be fresh. In the refrigerator (2-10°C), the sample loses up to 10% of the enzymatic activity in one day.

### REQUIRED MATERIAL (non-provided)

- Spectrophotometer.

- Micropipettes and pipettes for measuring the stated volumes

- Water bath at the temperature indicated under PROCEDURE.

- Stopwatch.

### ASSAY CONDITIONS

(Increase of Absorbance)

- Wavelength: 340 nm (Hg 334 or 366).
- Reaction temperature: 25, 30 or 37°C. Select temperature according to instrument. See the REFERENCE VALUES corresponding to each temperature.
- Reaction time: 15 minutes
- Sample and reagent volumes: may vary proportionally (e.g. 40 ul sample + 1 ml reconstituted Reagent A or 20 ul sample + 500 ul reconstituted Reagent A).

### PROCEDURE

Set the instrument to zero O.D. with distilled water. See INDICATION OF INSTABILITY OR DETERIORATION OF REAGENTS. In a cuvette at the selected temperature (25, 30 or 37°C) place:

Reconstituted Reagent A	2.5 ml
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Pre-incubate a few minutes. Then add:

Sample	100 ul
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Mix immediately by inversion. Wait 10 minutes. Adjust absorbance to a reference reading and simultaneously start stopwatch. Record absorbance every minute for 5 minutes. Determine average change in Absorbance/min ( $\Delta A/\text{min}$ ), subtracting each reading from the previous one and averaging these values. Use this mean for calculations.

### CALCULATIONS

CK MB (U/l) =  $\Delta A/\text{min} \times \text{factor}$

Measure at 340 nm: CK-MB (U/l) =  $\Delta A/\text{min} \times 8,254$

Measure at Hg 334 : CK-MB (U/l) =  $\Delta A/\text{min} \times 8,414$

Measure at Hg 366: CK-MB (U/l) =  $\Delta A/\text{min} \times 14,858$

The calculation factors above mentioned, already include the correction needed to convert the value of CK-B into CK-MB.

### QUALITY CONTROL METHOD

Each time the test is performed, analyze two levels of a quality control material (**CK-MB Control**) with known CK-MB activities.

### REFERENCE VALUES

Temperature	25°C	30°C	37°C
Values	10 U/l	16 U/l	25 U/l

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

### SI SYSTEM UNITS CONVERSION

CK-MB (U/l)  $\times 0.01$  = CK-MB (ukat/l)

### INTERPRETATION OF RESULTS

A high probability of myocardial damage exists if the following conditions are simultaneously met:

1- Total CK activity exceeds the following normal ranges:

Temperature	25°C	30°C	37°C
Men	10-80 U/l	15-130 U/l	24-195 U/l
Women	10-70 U/l	15-110 U/l	24-170 U/l

The possibility of a recent infarction exists if myocardial damage is suspected and values are below the normal range. In

this case repeat testing after 4 hours.

2- CK-MB activity exceeds normal values. See REFERENCE VALUES.

3- The CK-MB percentage is found between the 6-20% of the total CK value.

If the percentage is below 6% there is probably damage to the skeletal muscle. If the percentage is over 20% of the total CK value the presence of a macro kind of CK (atypical CK) which is not inhibited by the anti-CK-M antibodies, can be suspected.

The atypical CK presence may be determined by:

- Persistence for more than 48 hours (the CK-MB decays approximately at 30-48 hours after the onset of the infarction).
- Stability when treating the sample at 40°C during 20 minutes.
- Electrophoretic analysis (a band between MM and MB isoenzymes is obtained).

### PROCEDURE LIMITATIONS

See Known interfering substances under SAMPLE.

Samples with total CK activity over 1000 U/l should be diluted with saline solution (0.9% sodium chloride). The obtained result should be multiplied by the dilution performed.

### PERFORMANCE

**a) Reproducibility:** when replicates of the same sample were simultaneously assayed, the following values were obtained:

Level	S.D.	C.V.
30 U/l	$\pm 1.7$ U/l	5.7 %
80 U/l	$\pm 3.0$ U/l	3.7 %

**b) Detection limit:** depends on the photometer used and wavelength. According to the required sensitivity, in spectrophotometer at 340 nm (with 1 cm optical length square cuvettes,  $\pm 2$  nm reproducibility,  $\leq 0.5\%$  stray light,  $\leq 8$  nm pathlength) for  $\Delta A/\text{min}$  of 0.001, the smallest detectable activity change will be of 8 U/l.

**c) Dynamic range:** the useful reading range is extended up to 0.100  $\Delta A/\text{min}$  (340/334/366 nm).

**d) Linearity:** the reaction is linear up to 800 U/l (at 37°C).

### WIENER LAB PROVIDES

- 19 x 2.5 ml, with provided Control (Cat. N° 1271352).

### REFERENCES

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# Symbols

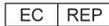
The following symbols are used in packaging for Wiener lab. diagnostic reagent kits.



This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices



Manufactured by:



Authorized representative in the European Community



Harmful



"In vitro" diagnostic medical device



Corrosive / Caustic



Contains sufficient for <n> tests



Irritant



Use by



Consult instructions for use



Temperature limitation (store at)



Do not freeze



Calibrator



Biological risks



Control



Volume after reconstitution



Positive Control



Contents



Negative Control



Batch code



Catalog number

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