



# CK - MB (NAC - act.)

E.C.2.7.3.2.

UV Method

Cat.No. 101-0005

Size 19 x 2.5 ml

## PRINCIPLE:

CK-MB is composed of the two moieties CK-M and CK-B. A specific antibody inhibits the CK-M moiety. This antibody will bind to and inhibit the activity of the M subunit of CK-MB. This means that only the activity of the B subunit in serum is measured. If the activity is multiplied by a factor of 2, it will give the activity of CK-MB in serum.

## SAMPLE:

Serum, plasma ( heparin, EDTA ).

## REAGENTS:

|   | Concentration in the test |
|---|---------------------------|
| 1. Buffer/Glucose (1 x 70 ml)                       |                           |
| Imidazole Buffer, pH 6.7                            | 100 mmol/L                |
| Glucose   | 20 mmol/L                 |
| Mg-Acetate  | 10 mmol/L                 |
| EDTA  | 2 mmol/L                  |
| 2. Enzymes/Coenzymes/Substrate/Antibody (19x2.5 ml) |                           |
| ADP   | 2 mmol/L                  |
| AMP   | 5 mmol/L                  |
| Diadenosine Pentaphosphate                          | 10 $\mu$ mol/L            |
| NADP  | 2 mmol/L                  |
| HK  | $\geq 2.5$ U/mL           |
| G <sub>6</sub> P-DH                                 | $\geq 1.5$ U/mL           |
| N-Acetylcysteine                                    | 20 mmol/L                 |
| Creatine Phosphate                                  | 30 mmol/L                 |
| Antibody to CK-M                                    |                           |
| 3. Control Serum (1 x 2 ml)                         |                           |

## REAGENT PREPARATION:

1. Buffer/Glucose  
Reagent ready for use; Stable up to the expiry date when stored at +2 °C to +8 °C.
2. Enzymes/Coenzymes/Substrate/Antibody  
Reconstitute one vial of Reagent 2 with 2.5 ml of buffer (Reagent 1.). The reconstituted reagent is stable 7 days at +2 °C to +8 °C or 12 hours at +15 °C to 25 °C.
3. Control Serum  
Open one vial of control serum very carefully, avoiding any loss of material, and reconstitute with exactly 2 ml redistilled water. Close vial carefully, let stand for 15 minutes, dissolving contents completely by swirling or rotating gently. CK-MB is stable in the serum for 5 days at +2 °C to +8 °C, 2 days at 25 °C and 4 weeks at - 20 °C.

## PROCEDURE:

Before carrying out the activity of CK-MB in serum, it is necessary to measure total CK activity using the CK-NAC method.

|              |                     |
|--------------|---------------------|
| Wavelength:  | 334, 340, 365 nm    |
| Cuvette:     | 1 cm light path     |
| Temperature: | 25 °C, 30 °C, 37 °C |
| Zero:        | air                 |

|   |        |
|---|--------|
| Pipette into working solution 2:  |        |
| Sample  | 0.1 ml |
| Mix, and let stand at the appropriate temperature for 10 minutes. Then read absorbance A <sub>1</sub> . |        |
| Read absorbance A <sub>2</sub> exactly 5 minutes later.   |        |

## CALCULATION:

$$\Delta A = A_2 - A_1$$

| Wavelength | CK-B U/L          | CK-MB U/L         |
|------------|-------------------|-------------------|
| 334 nm     | 841 x $\Delta A$  | 1683 x $\Delta A$ |
| 340 nm     | 825 x $\Delta A$  | 1651 x $\Delta A$ |
| 365 nm     | 1529 x $\Delta A$ | 3058 x $\Delta A$ |

## EXPECTED VALUES:

In serum taken from healthy people the CK-MB activities are below :

25 °C < 10 U/L (167 nkat/L)

30 °C < 16 U/L (267 nkat/L)

37 °C < 25 U/L (417 nkat/L)

Myocardial infarction (MI)

The likelihood of myocardial damage is high when the following 3 factors are present:

|   | 25 °C          | 30 °C           | 37 °C           | Units         |
|---|----------------|-----------------|-----------------|---------------|
| 1. CK (men)   | > 80<br>> 1334 | > 130<br>> 2167 | > 195<br>> 3251 | U/L<br>nkat/L |
| CK-MB (women)   | > 70<br>> 1167 | > 110<br>> 1834 | > 170<br>> 2834 | U/L<br>nkat/L |
| 2. CK-MB  | > 10<br>> 167  | > 16<br>> 267   | > 25<br>> 417   | U/L<br>nkat/L |
| 3. A CK-MB activity between 6 and 25% of the total CK activity. |                |                 |                 |               |

If MI is suspected but the values obtained are below the specified limits, a fresh infarct may have occurred. In this case, tests should be repeated after 4 hours.

## LINEARITY:

Antibody inhibits up to 2000 U/L (33340 nkat/L) of CK-MM.

## NOTE:

1. Solution 1 (buffer/glucose) contains sodium azide as preservative. Do not swallow. Avoid contact with the skin or mucous membranes.
2. Hemolytic sera must not be used for the determination of CK and CK-MB activities.
3. Greater accuracy is attained by measurement at 334 nm or 340 nm.
4. The absorbance change per minute (  $\Delta A/\text{min}$  ) may also be measured and the mean of 5 measurements is then used in the calculation ( result x 5 ) or the 5 measurements should be used in the calculation. The factors used will then become:

$$\Delta A \text{ 334 nm/min} \times 8414 = \text{U/L}$$

$$\Delta A \text{ 340 nm/min} \times 8254 = \text{U/L}$$

$$\Delta A \text{ 365 nm/min} \times 15294 = \text{U/L}$$

## REFERENCES :

1. Wurzburg, U. et al.: Clin.Chem. 54, 357 (1976)
2. Szasz,G., et al.: Paper presented at 3rd Eur. Congr. Clin. Chem., Brighton/England, June , 3-8, (1979) ( abstract )
3. Stein, W.: Med.Welt 36, 572 (1985)