ANA BIO CLR CK - MB

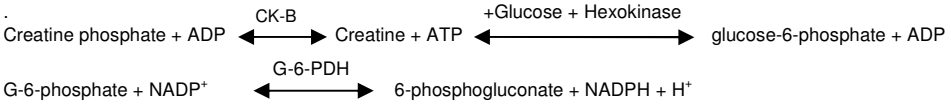
(Immunoinhibition method)

Intended Use

CK- NAC is a reagent set for determination of CK-MB activity in serum and plasma based on Immunoinhibition method.

Principle

The serum sample is incubated with CK-MB reagent containing antibody specific to CK-M subunit which completely inhibits the CK-M monomer. The activity of CK-B which is not inhibited by the antibody is then measured by the following reaction sequence.



Reagent provided

1. Reagent R1
2. Reagent R2

Working Reagent Preparation

Prepare working reagent by mixing Reagent R1 and Reagent R2 in the ratio 4:1 as per the number of tests required.

Reagent storage and stability

The reagent kit should be stored at 2-8℃ and is stable till the expiry date indicated on the label.
The working reagent (4 R1 + 1 R2) is stable for 10 days at 2-8℃.

Specimen collection and preservation

Blood should be collected in a clean dry container. Although serum is preferred, plasma with heparin or EDTA can be used. The assay should be carried out as far as possible on the same day. Serum and plasma samples are stable for 1 week at 4℃ and 1 month at -10℃. The samples should be brought to room temperature prior to use. Avoid use of haemolysed and grossly contaminated samples.

Assay guidelines for Analyzers

Reaction type	Kinetic Reaction
Reaction Slope	Increasing
Wavelength	340 nm
Flow cell Temperature	37℃
Delay time	300 seconds
Interval time	30 Seconds
No. of readings	4
Blank	Distilled water
Sample volume	50 µl (0.05 ml)
Working Reagent volume	1000 µl (1.0 ml)
Factor	6752
Linearity	Up to 2000 IU/l

Assay guidelines for Manual procedure

Pre warm the required amount of working reagent at 25℃ / 30℃ before use. Perform the assay as given below:

Reagents	**Standard**	**Test**
Working Reagent	1000 µl (1.0 ml)	1000 µl (1.0 ml)
Serum/Plasma	-	50 µl (0.05ml)

1. Mix thoroughly and transfer the assay mixture immediately to the thermo stated cuvette and start the stop watch simultaneously.
2. Record the first reading at 300th second and subsequently 4 more readings with 30 seconds interval at 340 nm.

Calculation

Activity of CK-MB In IU/l = ΔAbs/min. x 6752

Temperature Conversion:

Following factors can be used for conversion of IU/l from one temperature to another:

Assay Temperature	Desired temperature		
	25℃	30℃	37℃
25℃	1.00	1.53	2.38
30℃	0.65	1.00	1.56
37℃	0.42	0.64	1.00

Note: Since temperature conversion factors are given only as an approximate conversion, it is suggested that values be reported at the temperature of measurement.

Normal Range

	at 25℃	at 30℃	at 37℃
CK-MB	< 10 IU/l	< 15 IU/l	< 24 IU/l

Note: Expected range varies from population to population and each laboratory should establish its own normal range.

Limitations

Avoid using haemolysed serum since red blood cells may release enzymes and intermediates such as ATP and Glucose-6-phosphate dehydrogenase which may interfere in the assay. This procedure may over estimate CK-MB values if CK-BB activity in the serum is high. CK-BB activity is usually absent in sera from normal individual and patients with myocardial infarction. The presence of a macro form of CK-BB in the specimen should be suspected if the CK-MB activity measure by this procedure represents more than 20% of the total CK activity. The working solution is considered unsatisfactory and should not be used if the absorbance exceeds 0.700 at 340 nm against distilled water. If the CK-MB activity exceeds 2000 IU/L; then dilute the specimen suitably with normal saline and repeat the assay. In such case, the results obtained should be multiplied with the dilution factor to obtain correct CK-MB activity. The dilution of a sample that initially exceeds linearity often results in a higher than expected value; therefore instead of dilution it is recommended that a smaller sample volume be used.

Quality Control

To ensure adequate quality control, it is recommended that each batch should include a normal and an abnormal commercial reference control serum. It should be realized that the use of quality control material checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, and cleanliness of glassware and accuracy of pipetting.

References

1. Neumeier D,etal., Activity kinetics and Diagnostic Significance in Myocardial infarction**. Clinica Chimica Acta** 73; 445-451, 1976.
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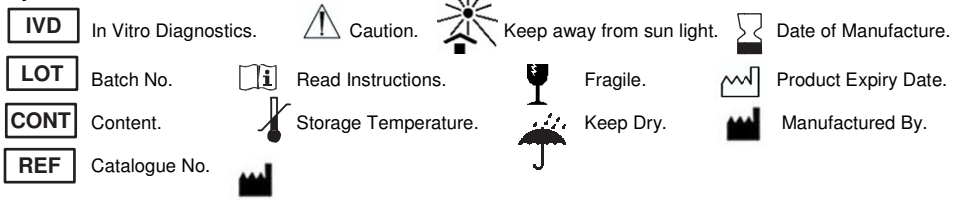
3. Melattini F, et al., **Clin. Chem**. 24/3: 498-501, 1978.
4. Wicks R, et al., Immunochemical Determination of CK-MB Isoenzyme in human serum, II. Enzyme approach **Clin. Chem**. 28/1; 54-58, 1982.
5. Tietz N. W., **Fundamentals of Clin. Chem**. (III) 377, 383 (1981).
6. Faulkner, Willard R., **Selcted Methods of Clin. Chem**. (9) 185 (1982).
7. In-house test data,

Symbols



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