ANA BIO ISP CK - MB

(Immunoinhibition Method)

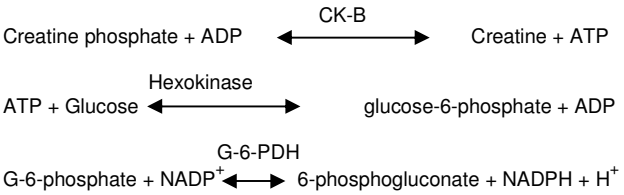
For Miura Instruments

Intended Use

CK- MB 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.



Components & Concentration of Reagents

Reagent	Component	Concentration
Reagent 1	Imidozole buffer pH 6.6	125 mmol/L
	HK	≥ 800 U/L
	Nac	25 mmol/L
	G6P-DH	≥ 800 U/L
	NADP	2.5 mmol/L
	CK-MM Ab	≥ 1000 U/L
	Mg Acetate	10 mmol/L
	Stabilizers, excipients & surface active agents	
Reagent 2	CP	30 mmol/L
	ADP	2 mmol/L
	AMP	6 mmol/L
	DAPP	0.010 mmol/L
	Stabilizers, excipients & surface active agents	

Reagent storage and stability

The reagent kit should be stored at 2° - 8°C and is stable till the expiry date indicated on the label.
A slight variation in the composition of the components may occur between batches, but this has no effect on the test results.
After opening, the vial R1 and R2 are stable 30 days if recapped immediately and protect from contamination, evaporation, direct light and stored at correct temperature.

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°C and 1 month at -10°C. The samples should be brought to room temperature prior to use. Avoid use of haemolysed and grossly contaminated samples.

Automation

This kit, though developed and manufactured to be used as manual assay and with I.S.E. Miura Analyzer, can be used also with other analyzers able to meet the specifications indicated in section "Reaction conditions – Test procedure" Application sheets are available for automatic instruments.
All applications not explicitly approved by KDPL. Cannot be guaranteed in terms of performance, and must there be established by the operator.

Calibration

For Calibration use the "Multicalibrator"

Calibration Stability

For the instrumentation series Miura, the calibration is recommended to be done every 10 days.

Materials required but not supplied in the kit

Calibrators and controls

Assay guidelines for Analyzer I.S.E. Miura

Analyte Name	CKMB	
Method Code	Ckmb	
Type	Kinetic-Substrate Start	
Unit	IU/L	
Filter F1	340 nm	
Blank in	Not Use	
Step	Reaction Volume	U.M.
Volume reagent R1	200	µl
Sample Volume	12	µl
Incubation R1, S → R2	240	Sec.
Volume reagent R2	50	µl
Final Incubation	180	Sec.
Kinetic reading	288	Sec.

Temperature Conversion:

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

Assay Temperature	Desired temperature		
	25°C	30°C	37°C
25°C	1.00	1.53	2.38
30°C	0.65	1.00	1.56
37°C	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°C	at 30°C	at 37°C
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 measures, 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, cleanliness of glassware, Wavelength setting, Expiration date of reagents and accuracy of prob aspiration.

Accuracy-Recovery

N= 96, R = 0.998, Y = 1.03x – 2.85

Interference
Triglycerides below 600 mg/dl does not interfere in the reaction. Haemoglobin interferes at concentrations above 50.0 g/L. Ascorbic Acid influences the reaction at concentrations over 64 mg/dl.

Precision of the Method

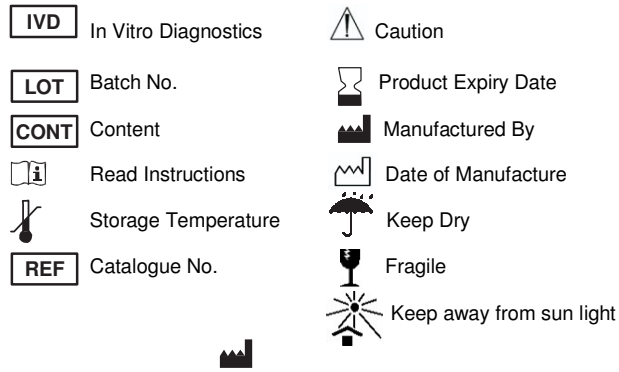
Within-run					
Range	U.M	Mean	S.D.	C.V.(%)	No. run
Low	IU/L	33.7	0.52	2.3	20
High	IU/L	125	2.15	1.8	20
Between run					
Range	U.M	Mean	S.D.	C.V.(%)	No. run
Low	IU/L	40	0.75	3.9	20
High	IU/L	135.5	2.20	1.8	20

Sensitivity
At 340 nm, the activity of CK-MB of 3 IU/L can estimate.

References

1. Neumeier D,etal., Activity kinetics and Diagnostic Significance in Myocardial infarction**. Clinica Chimica Acta** 73; 445-451, 1976.
2. Gerhardt W, et al., Creatine kinase B-sub unit Activity in human serum. **Clinica Chimica Acta** 78:29-41, 1977.
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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