CK –MB LYPHO

(Immunoinhibition method)

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.

.

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow{\text{CK-B}} \text{Creatine} + \text{ATP} \xrightarrow{\text{+Glucose+ Hexokinase}} \text{glucose-6-phosphate} + \text{ADP}$$

$$\text{G-6-phosphate} + \text{NADP}^{+} \xrightarrow{\text{G-6-PDH}} \text{6-phosphogluconate} + \text{NADPH} + \text{H}^{+}$$

Reagent provided

1. Reagent R1Enzyme
2. Reagent R2Buffer

Working Reagent preparation.

Reconstitute the Enzyme vial **(R1)** with the exact volume of **Buffer (R2**) mentioned on the enzyme vial label. Swirl gently to dissolvecompletely. Keep it for 10 minutes to stabilize before use.

Stability and storage.

Prior to use:

When stored between 2-8°C the unopened reagents are stable until the expiration date stated on the bottle and kit box label.

Working Reagent:

The working reagent is stable for 14 days when stored capped at 2-8°C or 2 days at room temperature (18-25°C).

Indications of Reagent Deterioration:

• Turbidity; The dry reagent should have a white to off white Appearance and should be freeflow.
• Working Reagent absorbance > 0.700 Abs at 340nm
• Failure to recover control values within the assigned range.

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.

Assay guidelines for Analyzers

Reaction type	Kinetic Reaction
Reaction Slope	Increasing
Wavelength	340 nm
Flow cell Temperature	37°C
Delay time	300 seconds
Interval time	30 Seconds
No. of readings	3
Blank	Distilled water
Sample volume	50 µl (0.05 ml)
Working Reagent volume	1000 µl (1.0 ml)
Factor	6752
Linearity	Up to 1500 IU/l

Assay guidelines for Manual procedure

Reagents	**Standard**	**Test**
Working Reagent	1000 µl (1.0 ml)	1000 µl (1.0 ml)

KE DIAGNOSTICS

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 3 more readings with 30 seconds interval at 340 nm.

Calculation

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

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 hemolysis 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 1500 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**. ClinicaChimicaActa** 73; 445-451, 1976.
2. Gerhardt W, et al., Creatine kinase B-sub unit Activity in human serum.**ClinicaChimicaActa**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 ofClin. Chem**. (III) 377, 383 (1981).
6. Faulkner, Willard R., **Selcted Methods ofClin. Chem**. (9) 185 (1982).
7. In-house test data,

Symbols

IVD In Vitro Diagnostics. | Caution. | Keep away from sun light. | Date of Manufacture.

LOT Batch No. | Read Instructions. | Fragile. | Product Expiry Date.

CONT Content. | Storage Temperature. | Keep Dry. | Manufactured By.

REF Catalogue No.

KEE DIAGNOSTICS PVT LTD
CIN: U24231DL2004PTC128343 .
A subsidiary of KEE PHARMA LTD
A-8 . 3RD FLOOR. NARAINA INDUSTRIAL AREA, PHASE 2.
NEW DELHI-110028.
W. www.keediagnostics.com.
E. info@keediagnostics.com, T. 011 43136000.

Kee Diagnostics
because life matters

CE **ISO 9001 : 2015 & ISO 13485 : 2016 Certified Company**

KEE DIAGNOSTICS