CK-MB
(IFCC UV Kinetic method)

MONOTEST

DIATEK

CLINICAL SIGNIFICANCE :

CK-MB is present in low concentration in normal human serum but is increased as a result of heart injury, and rarely, skeletal muscle damage. CK-MB is widely used as an indicator of acute myocardial infarction as the detection of elevated activities is considered highly specific for this condition.

TEST PRINCIPLE :

The procedure involves measurement of CK activity in the presence of an antibody to CK-M monomer. This antibody completely inhibits the activity of CK-MM and half of the activity of CK-MB while not affecting the B subunit activity of CKMB and CK-BB. Then we use the CK method to quantitatively determine CK-B activity. In serum, the CK-BB activity is minimal and can be ignored. The CK-MB activity is obtained by multiplying the CK-B activity by two.

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow{\text{CK}} \text{Creatine} + \text{ATP}$$

$$\text{ATP} + \text{D-Glucose} \xrightarrow{\text{HK}} \text{G-6-P} + \text{ADP}$$

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

CK = Creatine kinase
HK = Hexokinase
G-6-P = D-Glucose-6-phosphate
G-6-PDH = Glucose-6-phosphate dehydrogenase

The increasing absorbance of NADPH is measured photometrically following time and temperature controlled kinetic reaction.

REAGENTS COMPOSITION :

Concentrations in the working reagent (pH 6.7) :
Imidazole 100 mmol/L, D-Glucose 20 mmol/L
N-Acetyl-L-Cysteine 20 mmol/L, Magnesium acetate 10 mmol/L
EDTA 2 mmol/L, NADP 2 mmol/L, Hexokinase 2500 U/L
Creatine phosphate 30 mmol/L, ADP 2 mmol/L, AMP 5 mmol/L
Diadenosine pentaphosphate 10 µmol/L, G-6-PDH 1500 U/L
Anti-human polyclonal CK-M antibody (sheep) sufficient to inhibit up to 2000 U/L of CK-MM at 37 °C

KIT CONTENTS :

CODE No. CKMB01

Pack size :	(10 Test)
Reagent 1	10 Vials (Prefilled)
Reagent 2	1 x 1.1 ml

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2 - 8 ºC, and contaminations prevented during their use. Do not use reagents over the expiration date.

SAMPLES :
Serum or Heparinised plasma

MATERIALS REQUIRED BUT NOT PROVIDED :
CKMB Control (Use of assayed QC sera is recommended to validate test result).

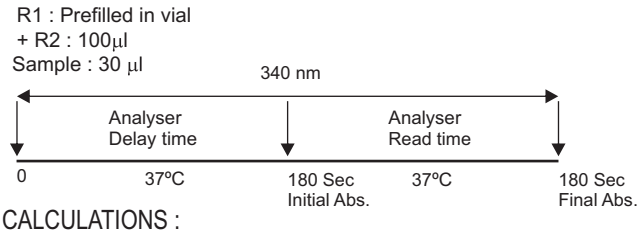
ASSAY CONDITIONS:

Cuvette:	1 cm light path
Wavelength :	340 nm
Constant temperature	37ºC
Reaction (Mode)	Kinetic
Kinetic Factor	6666
Delay	180 sec
Read time	180 sec
Linearity	1000 U/L
Unit	U/L
Blanking	D. Water
Slope of reaction	Increasing
Instrument sipping Volume	450 - 500 µl

PROCEDURE :

1. Add 100 µl of R2 directly into the prefilled R1 vial to prepare Working Reagent. Mix carefully (do not shake).
2. Programe the Analyser and set Sipping volume 450 - 500 µl.
3. Add 30 µl of sample in the Working Reagent.
4. Mix and start reading the change of absorbance (ΔA) with delay time 180 sec and Read time 180 sec at 37ºC.

Assay Procedure summary (Serum/Plasma):



CALCULATIONS :

CKMB Activity in U/L = Δ Abs/mint of Test x 6666

REFERENCE RANGE :

Serum/plasma : 0 - 24 U/L

The above reference range is guideline and all the laboratories must establish their own specific normal reference range. Final diagnosis should be made with correlation of clinical factors.

PRECAUTIONS :

1. Do not pipette by mouth therefore avoid contact of the reagent with skin.
2. Use fresh microtips while pipetting sample and R2
3. Avoid contamination of the reagent during the assay process.
4. **Before the assay begins, bring all the reagents to room temperature.**
5. Do not freeze or expose the reagents to high temperature and protect from direct sunlight as it will affect the performance of the kit.
6. Programmes for specific analysers are available on request.
7. For accuracy of results, the assay procedure, reagent preparation and storage has to be meticulously followed.
8. As with all the diagnostic procedures, the physician should evaluate data obtained by the use of this kit in light of other clinical information.

LINEARITY AND DETECTION LIMIT :

The linearity limit of the standard serum procedure is up to the CK activity of 1000 U/l. At higher activities the sample has to be diluted 1:10 with Normal saline and the final result to be multiplied by 10. The lowest detection limit for serum samples is equal to 5 U/L.

INTERFERENCES

The following analytes were tested up to the levels indicated and found not to interfere with Ascorbic acid upto 30 mg/dl, Bilirubin upto 40 mg/dl, Hemoglobin upto 200 mg/dl and Triglycerides upto 2000 mg/dl

The method will also measure any CK-BB isoenzyme present in serum. The activity of the isoenzyme is usually negligible, however, if a significant amount of CK-BB activity is present, the CK-MB activity will be overestimated.
A macro form of BB (immunoglobulin complexed) has been observed which will be measured as B in the assay. If the measured CK-B activity exceeds 20 % of the total CK activity, the presence of macro BB should be suspected.

BIBLIOGRAPHY

1. Stein W. Creatine kinase (total activity), creatine kinase isoenzymes and variants. In: Thomas L, ed. Clinical laboratory diagnostics. Frankfurt: TH-Books Verlagsgesellschaft;1998.p.71-80.

2. Moss DW, Henderson AR. Clinical enzymology. In:Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 617-721.

3. Würzburg U, Hennrich N, Orth HD, Lang H. Quantitative determination of creatine kinase I soenzyme catalytic concentrations in serum using immunological methods. J Clin Chem Clin Biochem 1977;15:131-7.

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