SPECTRUM

The Creative Approach to Bioscience

CREATINE KINASE MB (CK-MB)

REF: 239 001 (6 x 5 ml) 60 Test
REF: 239 002 (6 x 10 ml) 120 Test
REF: 239 003 (5 x 25 ml) 250 Test
REF: 239 004 (6 x 20 ml) 240 Test

SYMBOLS IN PRODUCT LABELLING

EC REP Authorised Representative	Use by/Expiration Date
IVD For in-vitro diagnostic use	CAUTION. Consult instructions for use
LOT Batch Code/Lot number	
REF Catalogue Number	Manufactured by
Consult instructions for use	(Xi) - Irritant
Temperature Limitation	

Intended Use

Spectrum Diagnostics Creatine Kinase MB (CK-MB) reagent is intended for the in-vitro quantitative, diagnostic determination of Creatine kinase MB in human serum on both automated and manual systems.

Background

Creatine kinase (CK) is an enzyme which is contained in heart, brain and skeletal muscles. Thus, an increase of circulating level of CK may be associated to myocardial infarction, acute cerebrovascular disease, trauma or diseases of skeletal muscles. After a myocardial infarct, CK level begins raising between 4th and 6th hour after first acute symptoms, reaching the peak between 18th and 30th hour and coming back to normal values during the 3rd day. CK is present in three different isoenzymatic forms, which could be separated by electrophoresis or column chromatography; each form is originated in different body tissues, paying off their diagnostic determinations. CK exists in serum in dimeric forms as CK-MM, CK-MB, and CK-BB and as macro-enzymes. Measurement of CK-MB is a quite specific test for detection of cardiac muscle damage and is therefore used for diagnosis and monitoring of myocardial infarction.

Method

After immunoinhibition with antibodies to the CK-M subunit, the CK-B activity is determined with a fixed rate method according to the recommendations of the International Federation of Clinical Chemistry (IFCC).

Assay Principle

A specific antibody inhibits the M subunits of CK-MM and CK-MB, and thus allows determination of the B subunit of CK-MB (assuming the absence of CK-BB or CK-1). CK-B catalytic concentration, which corresponds to half of CK-MB concentration, is determined from the rate of NADPH formation, measured at 340 nm, by means of the hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6PDH) coupled reactions1,3.

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

$$\text{ATP} + \text{Glucose} \xrightarrow{HK} \text{ADP} + \text{Glucose-6-phosphate}$$

$$\text{Glucose-6-phosphate} + \text{NADP}^+ \xrightarrow{G6PDH} \text{6-Phosphogluconate} + \text{NADPH} + \text{H}^+$$

Reagents

Reagent 1 (pH 6.7) (Buffer / Coenzyme)

Imidazol	125 mmol/L
D-Glucose	25 mmol/L
N-Acetyl-L-Cysteine	25 mmol/L
Magnesium acetate	12.5 mmol/L
NADP	2.5 mmol/L
EDTA	2 mmol/L

Reagent 2 (Enzymes)

ADP	15.2 mmol/L
AMP	25 mmol/L
P1,P5-di (adenosine-5'-) penta-phosphate	103 mmol/L
Glucose-6-phosphate Dehydrogenase (G6PDH)	9 KU/L
Creatine phosphate	250 mmol/L
Hexokinase (HK)	3 KU/L
Anti-human-CK-M.	

Precautions and Warnings

Do not ingest or inhalate. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

Deterioriation

Do not use Spectrum CKMB reagent in case of presence of particles or turbidity.

Reagent preparation, Storage and Stability

CK-MB reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles when properly stored refrigerated at 2 – 8 ^{o}C, protected from light and contaminations prevented during their use. Once opened, the reagent is stable for 2 months at the specified temperature.

Specimen Collection and Preservation

Serum free of hemolysis is the preferred specimen. Plasma containing heparin, EDTA, citrate or fluoride may produce unpredictable reaction rates. Stable for 2 hours at 20-25 ^{o}C, 5 days at 4-8 ^{o}C.Total CK concentration in the sample must be lower than 1000 U/L. Dilute the serum 1/2 if necessary, with NaCl (150 mmol/L).

System Parameters

Wavelength	340 nm (334-365 nm)
Optical path	1 cm
Assay type	Fixed rate
Direction	Increase
Sample: Reagent Ratio	1:25
Temperature	37 ^{o}C
Equilibration Time	60 seconds
Zero adjustment	against air
Sensitivity	2 U/L
Linearity	2000 U/L

Procedure

Pipette into a cuvette:

Reagent (R1)	400 µl
Reagent (R2)	100 µl

Mix well and incubate for 5 minute at 37 ^{o}C.

Specimen	20 µl

Read initial absorbance (A1) after 60 seconds and start timer simultaneously. Read (A2) after 5 minutes.

Calculation

$$(A2 - A1) \times 1651 = \text{U/L CKMB}$$

Units: One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions. The concentration is expressed in units per liter of sample (U/L).

Expected values

The discrimination value for myocardial infarction is around 25 U/L. However, an index higher than 6% of total CK concentration discriminates better. These values are for orientation purpose; each laboratory should establish its own reference range.

Quality Control

Normal and abnormal commercial control serum of known concentrations should be analyzed with each run.

Performance Characterstics

Precision

Within run (Repeatability)

	Level 1	Level 2
n	20	20
Mean (U/L)	45	129
CV%	3.5	3.2

Run to run (Reproducibility)

	Level 1	Level 2
n	20	20
Mean (U/L)	40	130
CV%	2.8	2.3

Methods Comparison

A comparison between Spectrum Diagnostics CK-MB reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.959 was obtained.

Sensitivity

When run as recommended, the minimum detection limit of the assay is 2.0 U/L.

Linearity

The reaction is linear up to CK-MB concentration of 2000 U/l; specimens showing higher concentration should be diluted 1+2 using physiological saline and repeat the assay (result×3).

Interferences:

Haemoglobin (< 2.5 g/L) ,lipemia (Lipids < 900 mg/dL) and Bilirubin (< 25 mg/dL) do not interfere. Presences in the sample of above normal concentrations of CK-BB or adenilate kinase, and of macro or mitochondrial CK interfere. Other drugs and substances may interfere[3,4].

Waste Disposal

This product is made to be used in professional laboratories.
Please consult local regulations for a correct waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental contamination.
S61: avoid release in environment. refer to special instructions/safety data sheets.

References

1. IFCC methods for the measurement of catalytic concentration of enzymes. Part 7: IFCC method for creatine kinase. JIFCC 1989; 1: 130-139.
2. Tietz Textbook of Clinical Chemistry, 3rd edition. Burtis CA, Ashwood ER. WB Saunders Co., 1999.
3. Friedman and Young. Effects of disease on clinical laboratory tests, 3th ed. AACC Press, 1997.
4. Urdal P and Landaas S. Clin Chem 1979; 25: 461-465.
5. Young DS. Effects of drugs on clinical laboratory tests, 3th ed. AACC Press, 1997.

ORDERING INFORMATION	
CATALOG NO.	**QUANTITY**
239 001	6 x 5 ml
239 002	6 x 10 ml
239 003	5 x 25 ml
239 004	6 x 20 ml

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