**Creatine Kinase MB (CK-MB)**

**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 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 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-I). 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 reactions 1,3.

$$\text{Creatine phosphokinase} + \text{ADP} \rightarrow \text{Creatine} + \text{ATP}$$

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

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

**Reagents**

**Reagent 1 (pH 6.7) (Buffer / Coenzyme)**

- Imidazole: 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 inhale. 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.

**Storage & Stability**

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use. Signs of reagent deterioration: Presence of particles and turbidity.

**Reagent preparation, Storage, and Stability**

- REF: 239 001 add 1 ml from R2 to one bottle of R1; mix gently
- REF: 239 002 add 2 ml from R2 to one bottle of R1; mix gently
- REF: 239 003 add 5 ml from R2 to one bottle of R1; mix gently
- REF: 239 004 add 4 ml from R2 to one bottle of R1; mix gently

Or prepare the working solution according to the number of test required by mixing 4 volumes of R1 with 1 volume of R2. Stability: 2 weeks at 2-8°C away from light sources.

**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 °C, 5 days at 4-8 °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: Kinetic
- Direction: Increase
- Sample: Reagent Ratio: 1:25
  - e.g.: Reagent volume: 1 ml
  - Sample volume: 40 μl
- Temperature: 37 °C
- Equilibration Time: 60 seconds
- Read time: 1 to 5 minutes
- Zero adjustment: against air
- Sensitivity: 2 U/L
- Linearity: 2000 U/L

**Procedure**

Pipette into a cuvette:

- Reagent (R1): 800 μl
- Reagent (R2): 200 μl

Mix well and incubate for 5 minute at 37 °C.

**Specimen**: 40 μl

Read initial absorbance after 60 seconds. and start timer simultaneously. Read again after 1, 2, 3, 4 and 5 minutes. Determine the mean absorbance change per minute (ΔA/min).

**Calculation**

$$\Delta A/\text{min} \times 8254 = \text{U/L CKMB}$$

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

Performance Characteristics

Precision

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<td>Mean (U/L)</td>
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<td>CV%</td>
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Run to run (Reproducibility)

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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.

References


ORDERING INFORMATION

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