HORIBA Instruments Incorporated
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Pointe Creatine Kinase – MB (CK – MB) Reagent Set

Intended Use
For the Quantitative Determination of Creatine Kinase-MB in Serum for Manual and/or Automated Procedures. **Rx Only.**

Summary and Principle
Creatine Kinase are dimeric molecules composed of M and B subunits and exist as the iso-enzymes MM, MB, and BB.[1] The subunits M and B are immunologically distinct. CK-MM and CK-MB are distributed primarily in the skeletal muscle and heart muscle, respectively, while CK-BB is present mainly in the brain and in tissues composed of smooth muscle.[2]

Following acute myocardial infarction, CK-MB activity increases significantly and this elevation is highly specific for the laboratory diagnosis of myocardial infarction.[3,4] Although total CK activity usually increases following myocardial infarction, in some patients only the CK-MB activity increases, while the total CK remains in the normal range.[5]

This method is an optimized UV-test according to DGKC (German Society of Clinical Chemistry) and IFCC (International Federation of Clinical Chemistry and Laboratory Medicine).

In this procedure CK activity is measured 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 CK-MB and CK-BB. Due to negligible concentrations of CK-BB in the circulation, the remaining activity, multiplied by a factor of 2, represents the activity of the CK-MB iso-enzyme.

Reagents
CK-MB (R1 Reagent)

Composition:	
Glucose	20.0 mmol/L
Magnesium Acetate	10.0 mmol/L
EDTA	2.0 mmol/L
Hexokinase	5.0 kU/L
LDH	1.5 kU/L
NAC	20.0 mmol/L
NADP	2.0 mmol/L
Imidazole Buffer	50.0 mmol/L
Monoclonal antibodies (mouse) against human CK-M, inhibiting capacity	> 2000 U/L

CK-MB (R2 Reagent)

Composition:	
ADP	10.0 mmol/L
AMP	20.0 mmol/L
Diadenosine pentaphosphate	50.0 µmol/L
Creatine phosphate	150.0 mmol/L
G6P-DH	20 kU/L
Imidazole Buffer	50.0 mmol/L

Precautions:
For In Vitro Diagnostic Use

Normal precautions exercised in handling laboratory reagents should be followed. Do not pipette by mouth. Reagents contain sodium azide which may be toxic if ingested. Sodium azide may also react with lead and copper plumbing to form highly explosive metal azides. Refer to Material Safety Data Sheet for any updated risk, hazard or safety information. Dispose of used or expired reagents according to your laboratory and governmental requirements.

Reagent Preparation: R1 and R2 liquid reagents are supplied ready-to-use for analyzers capable of dispensing 2 separate reagents. For analyzers not capable of 2 reagent dispensing or for manual use, prepare a Working Reagent in the ratio of 4 parts R1 Reagent to 1 part R2 Reagent (i.e., 24 mL R1 Reagent and 6 mL R2 Reagent).

Reagent Storage and Stability: Reagents are stable until the expiration date on their respective labels, when properly stored at 2-8°C and protected from light. Both the R1 and R2 Reagent should appear clear/colorless. Discard if either appears cloudy or contains particulate matter. Once prepared and protected from light, the Working Reagent is stable for 2 weeks at 2-8°C or 24 hours at 15-30°C.

Specimen Collection and Storage
All specimens used in this test should be considered potentially infectious. Universal precautions as they apply to your facility should be used for handling and disposal of materials during and after testing. Clear unhemolyzed serum is the specimen of choice. No special additives or preservatives are required. Whenever possible specimens should be separated and analyzed on the day of collection. Store serum in capped tubes. CK-MB activity in serum is reportedly stable for 4 weeks, when stored in a dark area at -20°C. Storing at other temperatures will result in a loss of activity; after 24 hours at 2-8°C, < 10%; after one hour at 15-30°C, < 10%. Extremely hemolyzed samples are not suitable for the test since they may contain high levels of adenylate kinase, ATP, and glucose-6- phosphate, which interfere with the assay to yield false results.

Interfering Substances
Ascorbic acid up to 30 mg/dL, conjugated bilirubin levels up to 24 mg/dL, unconjugated bilirubin levels up to 30 mg/dL and triglyceride levels up to 1000 mg/dL show no interference in this test. Hemoglobin interferes, even in minimal (25 mg/dL) concentrations.[8] Young et al[6] have reviewed drug effects on serum CK- MB levels. The described procedure may overestimate CK-MB values if CK-BB activity in the serum is very high. However, CK-BB activity is usually absent in sera from normal individuals and patients with myocardial infarction.[9] Some investigators have observed a macro form of BB (immunoglobulin complexed), which may be measured as B in this assay.[10,11,12] The presence of macro BB in the specimen should be suspected if the CK-B activity measured by this procedure represents more than 20% of the total CK activity.

Materials Provided
CK-MB R1 and R2 Reagents

Material Required But Not Provided
Spectrophotometer capable of absorbance reading at 340 nm and 1 cm lightpath, Constant temperature block or bath, 37°C, or temperature controlled cuvette Accurate pipetting devices, Test tubes, Interval timer

Automated Procedure
Applications for automated analyzers are available by contacting HORIBA Instruments Inc. Technical Support Department.

Manual Procedure
1. Allow reagents and specimens to equilibrate to ambient room temperature prior to use.
2. Prepare CK-MB Working Reagent according to instructions (see Reagent Preparation section).
3. Zero spectrophotometer at 340 nm with distilled water.
4. For each sample and control, add 1.0 mL Working Reagent to cuvette or test tube and incubate at 37°C for 4 minutes.
5. Add 40 µL of serum to its respective tube and mix gently.
6. Read and record absorbance at 5 minutes. Continue incubating at 37°C and record absorbance again at 6, 7, 8 and 9 minutes. Rate should be constant.
7. Determine the average absorbance per minute (ΔA/min), multiply by the factor 8360 (4180 x 2) for results in U/L.

NOTE: If cuvette is not temperature controlled, incubate samples at 37°C between readings.

Calibration
Calibration is not required. If calibration is required by the instrument manufacturer, follow the calibration guidelines to calibrate your analyzer.

Quality Control
HORIBA Medical recommends the use commercially available controls with CK-MB values assayed by this method for verifying accuracy and precision. Controls containing non-human CK-MB fractions are not suitable to be applied with this test due to the monoclonal antibody used in the reagent. Use controls containing exclusively human CK-MB. CK-MB activity determined in these materials, by this procedure should fall within the ranges for the controls. Two levels (Normal/Abnormal) of controls should be analyzed each day of testing.

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Results

CK-B Activity: Values are derived based on the absorptivity micromolar extinction coefficient of NADP at 340 nm (0.00622). A unit per liter (U/L) of CK-B activity is that amount of enzyme that oxidizes one µmol/L of NADP per minute.

$$\text{CK-B activity U/L} = \frac{\Delta A/\text{Min} \times \text{Total Volume (mL)}}{\text{Absorptivity Sample Volume (mL)}}$$

$$\text{CK-B activity U/L} = \frac{\Delta A/\text{Min}}{0.00622} \times \frac{1.040}{0.040}$$

$$\text{CK-B activity U/L} = \Delta A/\text{Min} \times 4180$$

$$\text{CK-MB Activity (U/L)} = \text{CK-B Activity (U/L)} \times 2$$

$$\%\ \text{CK-MB Activity} = \frac{\text{CK-MB Activity (U/L)} \times 100}{\text{Total CK Activity (U/L)}}$$

Limitations

If the ΔA/min. is greater than 0.345, dilute 1 part sample with 9 parts saline and re-assay. Multiply the results by 10. CK values for neonatal patients have not been established with this procedure.

Expected Values[7]

<24 U/L (37°C)**;** CK-MB activity is between 6 and 25 % of total CK activity. This range should serve only as a guideline. It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments, laboratories, and local populations.

Performance Characteristic[8]

Comparison: A group of 90 sera were assayed by the described CK-MB method and by a similar commercially available CK-MB reagent. Comparison of the results yielded a correlation coefficient of 1.00 and the regression equation was y = 1.00x + 2.08. Comparison studies were performed according to NCCLS Tentative Guideline, EP9-T.

Precision: Within-run precision was established by 20 assays on three different levels of commercial controls. Total precision values were obtained by assaying 3 commercial controls for 5 consecutive days.

Within-Run

	Serum 1	Serum 2	Serum 3
Mean CK-MB (U/L)	26.7	46.6	106
Std. Deviation	0.70	0.85	1.03
CV (%)	2.6	1.8	1.0

Total Precision

	Serum 1	Serum 2	Serum 3
Mean CK-MB (U/L)	28.2	52.7	109
Std. Deviation	1.05	1.66	2.32
CV (%)	3.7	3.2	2.1

Precision studies were performed according to NCCLS Tentative Guideline, EP5-T.

Linearity: Linear to 175 U/L at 37°C. Performed according to NCCLS Guideline EP6-P.

Sensitivity: Based on an instrument resolution of A=0.001, the method presented shows a sensitivity of 2.0 U/L.

References

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Symbol Key

Use by (YYYY-MM-DD)	LOT Lot and batch code
REF Catalog number	Manufacturer
IVD *In vitro* diagnostic medical device	Temperature limitation
Consult instructions for use	**Rx Only:** Prescription Use Only
CE mark	EC REP Authorized representative in the European Community

REF C7563 Manufactured for HORIBA Instruments Incorporated - Pointe Brand
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Certified to Perform Reagents

The Pointe reagents are certified to be manufactured according to specified parameters. Any Pointe reagent product not meeting specifications through its listed expiration date will be remedied immediately without charge.

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