Creatine Kinase-MB
Procedure No. FT702

For the Quantitative Determination
of Serum Creatine Kinase-MB (CK-MB)

Summary and Principle

Creatine Kinase are dimeric molecules composed of M and B subunits and exist as the isoenzymes 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]

In this procedure CK activity is measured in the presence of an antibody to CK-M monomer. This antibody completely inhibits the activity of CKMM 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 isoenzyme.

Reagents

CK-MB Reagent (powder), Cat. No.FT702a; 20vials for 6.5 ml each

ADP	2.0 mmol.L
G6PDH	2000 U/L
Creatine Phosphate	20 mmol/L
NAD	2.0 mmol/L
Hexokinase (yeast)	3000 U/L
D-Glucose	20 mmol/L

Anti-human CK-M Antibody (Goat) - Sufficient amount to inhibit up to 1500 U/L of CK-MM at 37˚ C. Also contains buffers, activators, surfactants and AK inhibitors.

Precautions: For in Vitro Diagnostic Use

Contains sodium azide. Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

Reagent Preparation: Reconstitute reagent with distilled water as indicated on vial label. Invert gently and swirl to dissolve completely.

Reagent Storage and Stability: CK-MB reagent is stable at refrigerated temperature (2-8˚ C) until the expiration date on the label. After reconstitution, the reagent is stable for 8 hours at room temperature or 30 days when stored at 2-8˚ C.

Materials Required But Not Provided

Spectrophotometer capable of absorbance readings at 340 nm.
Accurate pipetting devices.
Interval timer Cuvets Test Tubes
Constant temperature bath, or block, 37˚ C or temperature controlled cuvet well.
Mixer (Vortex type)

Specimen Collection and Preparation

Clear unhemolyzed serum is the recommended specimen sample. No special additives or preservatives are required.

Sample Stability: Serum CK appears stable for 3 days at 2-8˚ C. It is recommended that specimens be assayed immediately after collection. Reconstituted control sera may show a decrease in CK activity in only a few hours.

Interfering Substances: Extremely hemolyzed samples are not suitable for testing since they may contain high levels of adenylate kinase, ATP and glucose-6-phosphate which interfere with the assay to yield false results. Drugs and other substances which may interfere with the determination of CK activity have been listed by Young et al.[6] This procedure may overestimate CK-MB values if CK-BB activity in the serum is high. CK-BB activity is usually absent in sera from normal individuals and patients with myocardial infarction.[7] The presence of a macro form of CK-BB in the specimen should be suspected if the CK-MB activity measured by this procedure represents more than 20% of the total CK activity.

Automated Analyzer

Parameters:

Wavelength	340 nm
Reaction Type	Kinetic
Reaction Direction	Increasing
Reaction Temperature	37˚ C
Sample/Reagent Ratio	1:20
Equilibration Time	20 Seconds
Read Time	120 Seconds
Lag Time	300 Seconds
Blank Absorbance Limit	0.800A
High Absorbance Change/Min	...0.375A/Min.
Factor	3376
Low Normal	0 IU/L
High Normal	24 IU/L
Linearity	1500 IU/L

Above parameters should be employed in programming automated analyzers for CK-MB. Consult your instrument manual for programming instructions. Specific programming applications for most automated analyzers are available from Interchim Customer Service Department.

Manual Procedure

1. For each sample add 1.0 mL CK-MB reagent into a cuvet/test tube and warm for approximately 5 minutes at 37˚ C.
2. Add 50 uL of Sample to its respective tube, mix well and incubate for 5 minutes at 37˚ C.
3. Set the wavelength of the instrument at 340 nm. Zero the instrument with distilled water.
4. After the 5 minutes has elapsed, read and record the absorbance.
5. Record the increase in absorbance at 60 second intervals for the next 2 minutes. (ΔA/Min.). The rate of change should be constant.

NOTE: If cuvet/tube is not temperature controlled, incubate samples at 37˚ C between readings.

Quality Control: Two levels of control material with known CK/CK-MB levels determined by this method should be analyzed each day of testing. Interchim recommends the use of its Cardiac Isoenzyme Control Set; No. 0985, (Level I & II) for this purpose.

Results

A) **Total CK Activity**: Determine Total CK activity in serum according to the directions provided in the package insert for the CK reagent, Cat. No. FT7000.

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

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

$$\text{CK-B activity (U/L)} = \frac{\Delta A/\text{Min.}}{0.00622} \times \frac{1.050}{0.050}$$

$$\text{CK-B activity (U/L)} = \Delta A/\text{Min.} \times 3376$$

C) **CK-MB Activity (U/L)** = CK-B activity (U/L) x 2

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

NOTE: Samples having CK-MB values greater than 1500 U/L are diluted 1:2 (1 + 1) with distilled water, the assay repeated and results multiplied by the dilution factor of 2.

Temperature Conversion Factor

To convert CK-MB activity at 37˚C to 30˚C value, multiply the result by 0.60.

Expected Values[8]

0 - 24 U/L (37˚ C)
0 - 14 U/L (30˚ C)
% CK-MB < 5.5 %

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 Characteristics

Precision: Two pools were subjected to analysis over five consecutive days to determine total precision. Another two pools were subjected to triplicate analysis daily over a five day period to determine within run data.

The data follows:

Total Precision:

Pool	1	2
X	32	1228
SD	3.1	9.2
CV	9.8%	7.4%

Within Run:

Pool	1	2
X	34	132
SD	2.8	9.9
CV	8.2%	7.5%

Sensitivity: Based on an instrument resolution of A = 0.001, this procedure has a sensitivity of 4 U/L.

Correlation: Serum CK-MB determinations by the procedure described (y) and by a similar method (Sigma) (x) on 40 sera, showed a correlation coefficient (r) of 0.991 and a regression equation of y = 0.98x - 0.823.

Linearity: This procedure is linear to 1500 U/L. Samples having values greater than 1500 U/L should be diluted and reassayed.

References

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