

Creatine Kinase – MB (CK – MB) Reagent Set

Intended Use

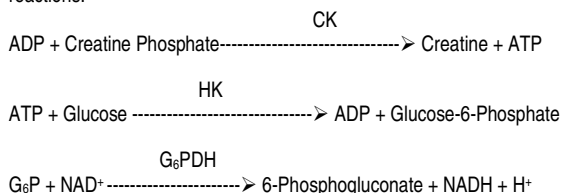
The CK-MB Reagent is intended to measure the activity of the isoenzyme CK-MB in human serum at 340nm.

Test Summary & Clinical Significance

Creatine kinase (EC 2.7.3.2) exists as dimers formed by two subunits that derive primarily from either muscle (M) or nerve cells (B). The combination of these subunits produces three isoenzymes of CK.^{1,2} CK-MM and CK-MB are found primarily in skeletal and heart muscle, respectively, while CK-BB is found mainly in the brain and smooth muscle tissue. Most CK activity in normal serum is due to the presence of the CK-MM isoenzyme. CK-MM activity increases following exercise, muscle trauma, shock and major surgery. The CK-BB isoenzyme is usually present in serum at very low activities and has been found to be rather unstable. Its activity increases in brain damage, malignant neoplasms, liver metastases and damage to the pregnant uterus.³ CK-MB is also 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.⁴ Conventional methods for the separation and quantitation of CK isoenzymes have been based on electrophoresis⁵ and ion-exchange chromatography.⁶ While these procedures can be useful, newer methods have recently been introduced by Wuerzburg et al⁷ and Gerhardt et al.⁸ Their procedure employs a polyclonal antibody to the CK-M monomer to completely inhibit the activity of CK-MM and one-half the activity of CK-MB. The activity of the non-inhibited CK-B monomer can then be measured. The present reagent includes the polyclonal antibody in a reagent that measures CK activity. Inhibition of CK-M occurs during the lag phase of the CK assay system, leaving only CK-B activity to be measured. The CK measuring reagent is based on a modification of the IFCC recommended formulation.

Principle

The sample is incubated in the CK-MB reagent that includes the anti-CK-M antibody. The activity of the non-inhibited CK-B is then determined using the following series of reactions:



The remaining CK-B catalyzes the reversible phosphorylation of ADP, in the presence of creatine phosphate, to form ATP and creatine. The auxiliary enzyme hexokinase (HK) catalyzes the phosphorylation of glucose by the ATP formed, to produce ADP and glucose-6-phosphate (G₆P). The G₆P is oxidized to 6-Phosphogluconate with the concomitant production of NADH. The rate of NADH formation, measured at 340nm, is directly proportional to serum CK-B activity.

Reagents

- CK-MB Reagent: (Concentrations refer to reconstituted reagent).
Creatine phosphate 30 mmol/L, ADP 2 mmol/L, Magnesium salt 10 mmol/L, D-glucose 20 mmol/L, AMP 5 mmol/L, NAD 2 mmol/L, Diadenosine pentaphosphate 10 umol/L, NAC 20 mmol/L, EDTA 2 mmol/L, Hexokinase (microbial) > 3300 U/L, G₆PDH (microbial)>2500 U/L, pH = 6.7 ±0.1, Non-reactive stabilizers and fillers.
- CK-MB Diluent: Buffer 100 mmol/L, Anti-human polyclonal CK-M antibody (from goat) sufficient to inhibit up to 2000 U/L of CK-MM at 37°C. Surfactant and sodium azide (0.1%) as preservative.

Reagent Preparation

Reconstitute each vial of CK-MB reagent with the volume CK-MB diluent specified on the vial label. Swirl to dissolve.

Reagent Storage and Stability

The unreconstituted reagent and diluent should be stored refrigerated (2-8°C). They are stable until the expiration date.

The reconstituted reagent is stable for at least six days in the refrigerator (2-8°C) and 24 hours at room temperature (15-30°C.)

Reagent Deterioration

Do not use if:

- Moisture has entered the vial and caking has occurred.
- The reconstituted reagent has an absorbance greater than 0.700 at 340 nm against water.

Precautions

The reagent contains sodium azide. This compound may react with lead and copper plumbing fixtures giving rise to explosive metal azides. Flush with large volumes of water when disposing of the reagent. All specimens and controls should be handled as potentially infectious, using safe laboratory procedures.

Specimen Collection and Storage

Serum is the specimen of choice for this assay. Avoid exposure of samples to strong light. Store samples in refrigerator (2-8°C), but no longer than one week. Freezing of samples (-20°C) results in minimal loss of activity.⁹

Interferences

Hemolyzed samples should not be used since erythrocytes contain contaminants and enzymes that will interfere with the assay. Bilirubin levels up to 20 mg/dl have been found to have a negligible effect on this assay.

Young et al¹⁰ lists a number of substances that will affect the assay. The method will also measure any CK-BB isoenzyme present in serum. The activity of this isoenzyme is usually negligible, however, if a significant amount of CK-BB activity is present the CK-MB activity will be overestimated.¹¹

A macro form of BB (immunoglobulin complexed) has been observed that will be measured as B in this assay. If the measured CK-B activity is greater than 20% of the total CK activity the presence of Macro BB should be suspected.^{12,13}

Materials Provided

CK-MB Reagent and Diluent

Materials Required but not Provided

- Spectrophotometer capable of accurate absorbance measurements at 340nm
- Matched cuvettes, 1 cm light path
- Constant temperature bath and thermal controlled spectrophotometer cell compartment
- Pipettes to measure diluent, reagents and samples

Procedure (Automated)

Wavelength:	340 nm
Assay Type:	Kinetic
Sample/Reagent Ratio:	1:20
Reaction Direction:	Increasing
Lag Phase:	300 seconds
Read Time:	60 seconds
Low Normal:	0 U/L
High Normal:	22 U/L

Application parameters for various automated instruments are available. Please contact our Technical Service Department for specific information.

Procedure (Manual)

- Determination of total CK activity
Determine the total activity of creatine kinase in the samples before carrying out the assay of CK-MB. Follow the procedure described in the direction insert for the total CK reagent. For proper comparison with the CK-MB assay, perform the assay of total CK at 37°C.
After determining the CK activity, dilute any sample with an activity higher than 2000 U/L at 37°C, using physiological saline.
- Determination of CK-B activity
Prepare the reagent as directed in the "Reagent Preparation" section. Bring needed amount of reagent to approximately 37°C.

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PROCEDURE:

1. Pipette 1.0 ml of CK-MB Reagent into a cuvette with 1 cm light path: one cuvette for the test and one cuvette for the blank.
2. Add 50 uL of sample to the test cuvette.
3. Add 50 uL of distilled or deionized water to the blank cuvette.
4. Mix both cuvettes well and incubate at 37°C for at least five minutes.
5. Measure the absorbance at 340 nm; set spectrophotometer to zero absorbance using water as a reference.
6. Read and record the change in absorbance per minute for three additional minutes.
7. Subtract the $\Delta A/\text{min}$ of the blank from the $\Delta A/\text{min}$ of the test to obtain the corrected $\Delta A/\text{min}$: $\Delta A/\text{min}(T) - \Delta A/\text{min}(B) = \Delta A/\text{min}(C)$

Limitations

1. If the assay of the total CK as reported above is greater than 2000 U/L at 37°C, dilute the sample appropriately with physiological saline before assay of CK-MB. Multiply result by the dilution factor to obtain the correct value of the isoenzyme.
2. The method will also measure any CK-BB isoenzyme present in serum. The activity of this isoenzyme is usually negligible, however if a significant amount of CK-BB is present, the CK-MB activity will be overestimated.¹¹
3. A macro form of BB (immunoglobulin complexed) has been observed that will be measured as B in this assay. If the measured CK-B activity is greater than 20% of the total CK activity, the presence of Macro BB should be suspected.^{12,13}

Calculations

$$\text{Corrected } \Delta A/\text{min} \times \frac{TV \times 1000}{6.22 \times LP \times SV} = \text{CK-B Activity (U/L)}$$

Where: TV = Total volume in mL (1.05 mL)
 SV = Sample volume in mL (0.05 mL)
 6.22 = Millimolar extinction coefficient of NADH
 1000 = To convert units per mL to units per L
 LP = Light path in cm (1 if a 1 cm cell was used)

$$\text{Then: } \Delta A/\text{min} \times \frac{1.05 \times 1000}{6.22 \times 1 \times 0.05} = \Delta A/\text{min} \times 3376 = \text{CK-B activity}$$

Multiply CK-B activity by 2 to obtain activity in terms of CK-MB (U/L).

Sample Calculation:

$\Delta A/\text{min}$ Sample = 0.0151
 $\Delta A/\text{min}$ Blank = 0.0001
 Corrected $\Delta A/\text{min}$ = 0.015

$$\text{CK-B activity} = 0.015 \times 3376 = 50.6 \text{ U/L}$$

$$\text{CK-MB activity} = 50.6 \times 2 = 101.2 \text{ U/L}$$

Percentage of CK-MB Activity in Sample:

This is calculated by dividing the value of CK-MB activity by the total CK activity and multiplying the ratio by 100 as follows:

$$\frac{\text{CK-MB Activity}}{\text{Total CK Activity}} \times 100 = \% \text{ CK-MB Activity}$$

Sample Calculation:

Total CK activity = 1250 U/L
 CK-MB Activity = 101 U/L

$$\% \text{ CK-MB Activity} = \frac{101}{1250} \times 100 = 8.1\%$$

Quality Control

Serum controls are recommended to monitor the performance of the assay procedure with every working shift in which CK-MB assays are performed. It is recommended that each laboratory establish their own frequency of control determination.

Expected Values

The range of values of CK-MB activity found in 132 apparently normal adult males and females was between 0 and 22 U/L at 37°C. A ratio between CK-MB and Total CK activities above 4% should be considered suspicious, even though it could be caused by extensive skeletal muscle injury. Any ratio between 5.5-20% is consistent with acute myocardial infarction.¹⁴ It is highly recommended that each laboratory establish its own expected range.

Performance

1. Correlation: Manual results obtained in 44 serum samples ranging from 9 to 264 U/L were compared with those obtained using a commercial reagent (RAI). The correlation coefficient was 0.997 and the regression equation was $y = 0.99x - 1.3$. Automated: 91 serum samples ranging from 6 to 756 U/L were compared with a similar reagent on an automated system. The correlation coefficient was 0.999 and the regression equation was $y = 1.07x - 4.4$.
2. Precision:

Within Run			Run to Run		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
19.1	0.46	2.4%	18.8	0.78	4.1%
42.4	0.55	1.3%	42.8	0.98	2.3%
116.4	2.25	1.9%	116.9	2.27	1.9%

3. Specificity: the ability of the polyclonal antibody to inhibit the M-subunit of human CK was determined by testing the activity of human CK-MM isoenzyme at a level over 2000 U/L (at 37°C). The CK-MM isoenzyme was inhibited > 99.5%.
4. Sensitivity: Using this reagent with the described procedure, a change in absorbance per minute of 0.001 at 340 nm is equivalent to 6.8 U/L of CK-MB activity.

References

1. Dawson, D.M., et al, Biochem Biophys Res Comm 21:346, 1965.
2. Orth, H.D., Creatine Kinase Isoenzymes. Springer Verlag, New York, pp. 10-18, 1981.
3. Lang, Hl, Wuerzburg, U., Clin. Chem. 1439, 1982.
4. Wagner, G.S., et al, Circulation 47:263, 1973.
5. Roberts, R., Henry, P.D., Witteveen, S.A., Sobel, B.E., Am. J., Cardiol., 33,650, 1974.
6. Mercer, D.W., Clin. Chem. 20, 36, 1974.
7. Wuerzburg, U., Heinrich, N., Lang, H., Prellwitz, W., Neumeier, D., Knedel, M., Klin. Wochenschr., 54, 357, 1976.
8. Gerhardt, W., Ljungdahl, L., Borjesson, J., Hofvendahl, S., Hedenas, B., Clin. Chem. Acta. 78, 29, 1977.
9. Pesce, A. J. and Kaplan, L.A., Methods in Clinical Chemistry. Editors. The C.V. Mosby Company, St. Louis, 1987.
10. Young, D.S., Effect of Drugs on Clinical Laboratory Tests. Third Edition. AACC press, Washington, D.C., 1990.
11. Jockers-Wretou, E., Pfledierer G., Clin Chem Acta 58:223, 1975.
12. Ljungdahl, L., Gerhardt, W., Clin Chem 24:832, 1978.
13. Urdal, P., Landaas, S., Clin Chem 25: 461, 1979.
14. Wu, A.H.B., and Bowers, G.N. Jr., Clin Chem. 28, 2017, 1982.

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