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CREATINE KINASE-MB (CK-MB) REAGENT SET

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For the quantitative determination of Creatine Kinase-MB activity in serum.

INTRODUCTION

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]

Conventionally, CK isoenzymes are quantitated after first separating the three species by either electrophoresis[6], column anion exchange[7], or batch anion exchange chromatography. However, these methods are time consuming. Recently, Wurzburg et al. have introduced an immunoinhibition method[8]. This methodology forms the basis of our CK-MB reagent.

PRINCIPLE

This procedure involves measurement of CK activity 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. Then we use the CK method to quantitatively determine CK-B activity. The CK-MB activity is obtained by multiplying the CK-B activity by two.

REAGENT COMPOSITION

The active ingredients in CK-MB Reagent, when reconstituted according to the directions, will have approximately the following concentrations:
Creatine Phosphate 20 mM; Adenosine-5'-Phosphate 2mM; Nicotinamide Adenine Dinucleotide (NAD) 2mM; Hexokinase (Yeast) ≥ 3000 U/L; Glucose-6-Phosphate Dehydrogenase (Bacterial) ≥ 2000 U/L; Anti-Human CK-M Antibody (Goat)-sufficient amount to inhibit up to 1500 U/L of CK-MM at 37°C.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic use.
2. Exercise the normal precautions required for the handling of all laboratory reagents. Pipetting by mouth is not recommended for any laboratory reagent.

REAGENT PREPARATION

Reconstitute with volume of distilled water specified on each vial, swirl to dissolve.

STORAGE AND STABILITY

The reagent should be stored at 2 - 8°C prior to reconstitution. The reagent may be used until the expiration date indicated on the package label. After reconstitution, the reagent is stable for eight (8) hours at room temperature or fourteen (14) days at refrigerator temperature.

SPECIMEN COLLECTION

The serum should be free of hemolysis.[9] Serum CK activity is reported to be stable for 24 hours at room temperature (18 - 26°C) and for 14 days refrigerated (2 - 6°C) or frozen (-20°C). It is recommended that specimens be assayed soon after collection.

INTERFERING SUBSTANCES

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. Drugs and other substances which may interfere with the determination of creatine kinase activity have been listed by Young et al.[10] 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[I1] . Some investigators have observed a macro form of BB (immunoglobulin complexed) which may be measured as B in this assay[12,13,14]. 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 REQUIRED BUT NOT PROVIDED

Sample and reagent pipettes, test vials or cuvettes, timer, thermoregulated flowcell, spectrophotometer, control serum.

PROCEDURE (AUTOMATED)

Consult our appropriate instrument application instructions.
Note: Certain instruments require different reconstitution volumes than those stated on the vial label. Refer to appropriate application sheets.

PROCEDURE (MANUAL)

1. Reconstitute CK-MB reagent according to instructions.
2. Pipet 1.0 ml of reagent into appropriate test tubes and pre-warm at 37°C for at least five (5) minutes.
3. Zero spectrophotometer with water at 340 nm.
4. Add 0.050 ml (50 µl) of sample to reagent, mix and incubate at 37°C for five (5) minutes.
5. After five (5) minutes, read and record the absorbance. Return tube to 37°C. Repeat readings every minute for the next two (2) minutes.
6. Calculate the average absorbance difference per minute (ΔAbs./min.).
7. The ΔAbs./min. multiplied by the factor 3376 (**see Calculations**) will yield CK-B results in IU/L.
8. Samples with values above 1500 IU/L should be diluted 1:1 with saline, re-assayed and the results multiplied by two.

NOTE:

If the spectrophotometer being used requires a final volume greater than 1.0 ml for accurate readings, 3.0 ml of reagent and 0.15 rnl (150 µl) of sample can be used.

If the spectrophotometer being used is equipped with a temperature controlled cuvette, the reaction mixture may be left in the cuvette while readings are taken.

PROCEDURE LIMITATIONS
The procedure assumes that CK-BB activity in the sample is negligible. If a significant amount of CK-BB activity is present, then the CK-MB activity will also be overestimated.

CALCULATIONS
A) Total CK Activity:
Determine Total CK Activity in serum according to the directions provided in the package insert for CK Reagent.

B) CK-B Activity:

$$IU/L = \frac{\Delta Abs./min. \times TV \times 1000}{d \times \in \times SV} = \frac{Abs./min. \times 1.050 \times 1000}{1 \times 6.22 \times 0.050}$$

$$= \Delta Abs./min. \times 3376$$

Where: ΔAbs./min. = Average absorbance change per minute
TV = Total reaction volume (1.050)
1000 = Conversion of IU/ml to IU/L
d = Light path in cm (1.0)
$\in$ = Millimolar absorptivity of NADH (6.22)
SV = Sample volume in ml (0.050)

C) CK-MB Activity:
CK-MB activity is calculated from CK-B activity as follows:

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

*CK-MB molecule is a dimmer consisting of a B subunit and an M subunit. Antibody complexing with the M subunit results in loss of half the catalytic activity of the CK-MB molecule. Therefore, CK-MB activity in the sample is equal to twice the CK-B activity.

EXAMPLE OF CALCULATION

If your average absorbance change per minute is 0.020 then 0.020 x 3376 = 67.5 IU/L (CK-B Activity).

NOTE: CK-MB activity (IU/L) = CK-B activity (IU/L) x 2

For example, if CK-B activity is 67.5 IU/L then CK-MB = $67.5 \times 2 = 135.0$

Percentage of CK-MB activity in sample is:

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

For example, if the total CK activity is 1007 IU/L, the CK- B activity is 67.5 IU/L, and the CK-MB activity is 135 IU/L then

$$\% \text{ CK-MB activity} = \frac{135 \times 100\%}{1007} = 13.5\%$$

CALIBRATION
The CK activity in the sample is calculated based on the millimolar absorptivity of NAD.
CK-MB reagent is suitable for CK isoenzyme assay when total CK activity in the sample does not exceed 1500 IU/L at 37°C.

QUALITY CONTROL
Use control sera with known normal and abnormal values to monitor the integrity of the reaction in each set of assay. Values should be acceptable for this method and temperature.

TEMPERATURE CONVERSION FACTORS
To convert CK-MB activity at 37°C to 30°C value, multiply the result by 0.60.

EXPECTED VALUES[15]
0-24 IU/L (37°C)
0-14 IU/L (30°C)
% CK-MB < 5.5%

It is strongly recommended that each laboratory establish its own normal range.

PERFORMANCE
Linearity: 1500 IU/L

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

Comparison: Studies done between this procedure and Sigma procedure yield a correlation coefficient of 0.98 with a regression equation of Y = 0.98X - 0.823 (N= 40).

Precision:

Within Run

Mean (IU/L)	S.D.	C.V.%
34	2.8	8.2
132	9.9	*7.5*

Run to Run

Mean (IU/L)	S.D.	C.V.(%)
32	3.1	9.8
122.8	9.2	7.4

REFERENCES

1. Dawson, D M, et al., *Biochem. Biophys. Res. Comm* 21: 346 (1965).
2. Neumeir D., *Tissue Specific Distribution of Creatine Kinase Isoenzyme*, H. Lang, Editor, Springer Verlag, New York, p 85-109 (1981).
3. Wagner, et al,, *Circulation*: 47:263 (1973).
4. Bais R., Crit Rev., *Clin. Lab Sci*, 18:291 (1982).
5. D'Souza JP et al., *Clin. Biochem*. 11:204 (1978).
6. Robert et al., *Am. J. Cardiol*. 33:650 (1974).
7. Mercer D.W., *Clin. Chem*. 20:36 (1974).
8. Gerhardt et al., *Clin. Chem. Acta* 78:29 (1977).
9. Kaehmar, J.F. and Moss, D.W.: *Fundamentals of Clinical Chemistry.* Tietz N.W. ed. Saunders, W.B. Co., Philadelphia, 686 (1976).
10. Young, D. S, et al., *Clin Chem* 21:10 (1975).
11. Lang, H et al., *Clin. Chem*. 28:1439 (1982).
12. Lott J.A., *Clin. Lab Med.* 6:547 (1986).
13. Ljungdahl 1., Gerhartdt W., "Creatine kinase isoenzyme variants in human serum." *Clin. Chem* 24:832, (1978).
14. Urdal P, Landaas S: "Macro-creatine kinase BB in serum, and some data on its prevalence.," *Clin. Chem* 25:461, (1979).
15. Wu AHB, Bowers CN Jr: "Evaluation and comparison of immunoinhibition and immunoprecipitation methods for differentiating MB from BB and macro forms of creatine kinase isoenzymes in patients and healthy individuals.," *Clin Chem*: 2017, (1982).

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