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CREATINE KINASE-MB (CK-MB) REAGENT SET

INTENDED USE
CK-MB is intended to measure the activity of isoenzyme CK-MB in human serum.

INTRODUCTION
Creatine kinases 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 conventional methods, CK isoenzymes are quantitated after first separating the three species by electrophoresis[6], column anion exchange[7], or batch anion exchange chromatography. However, these methods are time consuming. In recent times, Wurzburg et al. has introduced an immunoinhibition method[8]. This methodology forms the basis of our CK-MB reagent.

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

$$\text{ADP + Creatine Phosphate} \xrightarrow{\text{CK}} \text{Creatine + ATP}$$

$$\text{ATP + Glucose} \xrightarrow{\text{HK}} \text{ADP + Glucose-6-Phosphate}$$

$$\text{G-6-P} + \text{NAD}^+ \xrightarrow{\text{G6PDH}} \text{6-Phosphogluconate + NADH} + \text{H}^+$$

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 format, to produce ADP and glucose-6-phosphate (G-6-P) is oxidized to 6-phosphogluconate with the concomitant production of NADH. The rate of NADH formation, measured at 340 nm, is directly proportional to serum CK-B activity.

REAGENT COMPOSITION
1. CK-MB Reagent:
   Creatine Phosphate 30 mM; Adenosine-5'-Phosphate 2mM; Nicotinamide Adenine Dinucleotide (NAD) 2mM; Hexokinase (Yeast) ≥ 3000 U/L; Glucose-6-Phosphate Dehydrogenase (Bacterial) ≥ 2000 U/L
2. CK-MB Diluent:
   Buffer 100 mM, 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 each vial of CK-MB reagent with the volume CK-MB diluent specified on the vial label. Swirl to dissolve.

STORAGE AND STABILITY
The unreconstituted reagent and diluent should be stored at 2 - 8°C. They are stable until the expiration date. The reconstituted reagent is stable for at least 7 days in refrigerator (2 - 8°C) and 24 hours at room temperature (15 - 30°C).

SPECIMEN COLLECTION
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.

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[11]. 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 tubes or cuvettes, timer, thermoregulated flowcell, spectrophotometer, control serum.

AUTOMATED PROCEDURE
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.

MANUAL PROCEDURE
1. Reconstitute CK-MB reagent according to instructions.
2. Pipette 1.0 ml of CK-MB reagent into the appropriate test tubes and pre-warm at 37°C for at least two (2) minutes.
3. Zero spectrophotometer with water at 340 nm.
4. Add 0.050 ml (50µl) of sample to the reagent, mix, and incubate at 37°C for five (5) minutes.
5. After five minutes, read and record the change in absorbance per minute for 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 (2).

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 ml (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.

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

1. Total CK Activity:
   Determine total CK Activity in serum according to the directions provided in the package insert for CK Reagent.

2. CK-B Activity:

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

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

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)
∈	=	Millimolar absorptivity of NADH (6.22)
SV	=	Sample volume in ml (0.050)

3. 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 dimer 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:
If your average absorbance change per minute is 0.020, then:
0.020 × 3376 = 67.5 IU/L (CK-B Activity)

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

For example, if CK-B activity is 67.5 IU/L then
CK-MB = 67.5 × 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 .60.

EXPECTED VALUES[15]

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

It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments, laboratories, and local populations.

PERFORMANCE CHARACTERISTICS

1. *Linearity:* 1500 IU/L
2. *Sensitivity:* Based on an instrument resolution of A = 0.001, this procedure has a sensitivity of 4 IU/L.
3. *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).
4. *Precision:*

		Within Run	
	Mean (IU/L)	S.D.	C.V. (%)
Normal	34	2.8	8.2
Abnormal	132	9.9	7.5

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

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