D-TEK

CREATINE KINASE – MB (CK-MB) REAGENT

Cat : DT525 – 30/60

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

INTRODUCTION
Creatine Kinase are dimeric molecules composed of M and B subunits and exist as the isoenzymes MM, MB, and BB. 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. Following acute myocardial infarction, CK-MB activity increases significantly and this elevation is highly specific for the laboratory diagnosis of myocardial infarction. 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.

Conventionally, CK isoenzymes are quantitated after first separating the three species by either electrophoresis, column anion exchange, or batch anion exchange chromatography. However, these methods are time consuming. Recently, Wurzburg et al. have introduced an immunoinhibition method. 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 non-inhibited CK-B is then measured using the following series of reactions:

CK
ADP + Creatine Phosphate$\rightleftharpoons$Creatine + ATP
HK
ATP + Glucose$\rightleftharpoons$ADP + Glucose 6 Phosphate
G6PDH
G-6-P + NAD$^+$ ------------>6-Phosphogluconate + NADH + 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 oxidised 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.

REAGENT COMPOSITION
CK MB Reagent
The active ingredients in CK-MB Reagent, when reconstituted according to the directions, will have approximately the following concentrations:
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;

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 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 twenty four (24) hours at room temperature 15-30°C or seven (7) days in refrigerator 2 - 8°C.

SPECIMEN COLLECTION
The serum should be free of hemolysis. Serum CK activity is reported to be stable for 7 days refrigerated (2 - 8°C). Freezing of samples (-20°C) results in minimal loss of activity. It is recommended that specimens be assayed soon after collection. Avoid exposure of samples to direct light.

INTERFERING SUBSTANCES
Extremely hemolyzed samples are not suitable for the test since they may contain high levels of adenyate 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 Youngh et al.

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 two (2) minutes.
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 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.

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:

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)
ɛ = 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:

\[
\text{CK-MB Activity (U/L)} = \frac{\text{CK-B Activity (U/L)}}{2}
\]

*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 OF CALCULATION
If your average absorbance change per minute is 0.021 then 0.021 x 3376 = 70.9 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 70.9 IU/L then CK-MB = 70.90 x 2 = 141.80

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 1012 IU/L, the CK-B activity is 70.90 IU/L, and the CK-MB activity is 141.80 IU/L then

\[
\% \text{ CK-MB activity} = \frac{141.8 \times 100}{1012} = 14.0 \%
\]

CALIBRATION
The CK activity in the sample is calculated based on the millimolar absorbptivity 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

- 0-24 IU/L (37°C)
- 0-14 IU/L (30°C)

% CK-MB < 5.6%

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

PERFORMANCE
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:

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<th>Mean (IU/L)</th>
<th>S.D.</th>
<th>C.V.%</th>
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<td>34</td>
<td>2.8</td>
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<td>132</td>
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REFERENCES

Date revised: 07/06