Creatine kinase (CK-MB) INTENDED USE

CK-MB (MB) reagent is an immunoinhibition assay intended for the in vitro quantitative determination of Creatine Kinase (CK-MB) in human serum and plasma.

CLINICAL SIGNIFICANCE

Creatine kinase (ATP: Creatine N-phosphotransferase, EC2.7.3.2) is a dimeric enzyme composed of two types of monomer subunits, M (Muscular) and B (Brain). The subunits combine to form three distinct CK isoenzymes, CK-BB (CK-1), CK-MB (CK-2) and CK-MM (CK-3). CK-MM is the predominant form of CK in skeletal muscle. CK-BB is found in cardiac and smooth muscle. CK-MB is found in heart and brain smooth muscle. Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range. For diagnostic purposes, the CK-MB results should always be assessed in conjunction with the patient’s medical history, clinical examination and other findings.

METHOD

Kinetic UV method according to IFCC specifications. Liquid stable reagent.

ASSESS PRINCIPLE

Creatine Kinase is a dimer. Its monomeric subunits are designated M (muscle) and B (brain, nerve cells). The subunits combine to form three isoenzymes namely CK-BB, CK-MB and CK-MM. The reagent contains a monoclonal antibody mix to the CK-M monomer and so completely inhibits the activity of CK-M and one half of the activity of CK-MB. The activity of the non-inhibited B monomer subunit of CK-MB is measured which represents half the activity of CK-MB. The method assumes that the activity of CK-BB isoenzyme in serum is essentially zero. In this method serum is added to a modified CK-MB reagent containing a monoclonal antibody mix to the MB isoenzyme in serum. The activity of CK-MB is measured which represents half the activity of CK-MB-M. The method assumes that the activity of CK-BB isoenzyme in serum is essentially zero. In this method serum is added to a modified CK-MB reagent containing a monoclonal antibody mix to the MB isoenzyme in serum. The activity of CK-MB is measured which represents half the activity of CK-MB.

EXPECTED VALUES

Normal < 24 U/L

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range. For diagnostic purposes, the CK-MB results should always be assessed in conjunction with the patient’s medical history, clinical examination and other findings.

REAGENTS

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Concentration (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>20</td>
</tr>
<tr>
<td>NADP</td>
<td>10</td>
</tr>
<tr>
<td>ADP</td>
<td>2.5</td>
</tr>
<tr>
<td>Hexokinase</td>
<td>4 KU</td>
</tr>
<tr>
<td>EDTA</td>
<td>2 mmol/l</td>
</tr>
<tr>
<td>Creatine phosphate</td>
<td>30 mmol/l</td>
</tr>
<tr>
<td>Diadenosine-5'-pentaphosphate</td>
<td>10 μmol/l</td>
</tr>
<tr>
<td>G6PDH</td>
<td>1.5 KU</td>
</tr>
<tr>
<td>CK-M inhibiting polyclonal antibodies</td>
<td>2000 U/L</td>
</tr>
</tbody>
</table>

REAGENT PREPARATION & STABILITY

All reagents are ready for use and stable up to the expiry date given on label when stored at 2–8°C. Mix 4 ml of R1 + 0.1 ml of R2 Mix well, do not shake, the working solution is stable for:
- 2 days at 15–25°C
- 2 weeks at 2–8°C

The rate of reduction of the enzyme NADP is proportional to the CPK activity in the specimen. It is determined by measuring the increase in absorbance at 334 / 340 / 365 nm correspondingly.

PROCEDURE

1. Pipette into test tube or cuvette
2. Mix
3. Work solution for 1 min
4. Test for 3 min
5. Incubate simultaneously
6. Read again after exactly 1, 2, and 3 minutes.

CALCULATION

Determine the change in absorbance per minute (AU/min) from the linear portion of the reaction curve and calculate the CK-MB concentration by using the following formula:

\[
\text{CK-MB (U/l)} = \Delta A \times X \text{ Factor}
\]

Factor for 340 nm: 8254

\[
\text{Factor} = \frac{\Delta A \times X \times 2 \times TV \times 1000}{SV \times \Sigma \times \text{LP}}
\]

Where:
- TV: Total reaction volume in ml
- SV: Sample volume in ml
- Σ: millimolar absorptivity of NADH
- LP: Cuvette pathlength in cm
- 1000: Conversion of U/ml to U/l

Multiplication of the CK-MB value by 2 gives an estimation of the CK-MB activity as half the activity is measured.

● Reagent Preparation & Stability

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- 2 days at 15–25°C
- 2 weeks at 2–8°C

● Specimen Preparation & Stability

Separate serum or clot from cells immediately. CPK is stable for 8 days at 2 – 8°C or one month stored at -20°C

● Manual Procedure

Vitro Scient, Industrial Area, Basatin Al Ismailia, Belbis, Alsharkia, Egypt.
Tel. +20552642664; Email: info@vitroscient.com; Website: www.vitroscient.com
It is recommended that controls (normal and abnormal) be included in:
- Each set of assays, or
- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Commerically available control material with established CPK-MB values may be routinely used for quality control. Failure to obtain the proper range of values in the assay of control material may indicate:
- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

The following corrective actions are recommended in such situations:
- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact Vitro Technical Services.

INTERFERING SUBSTANCES

- Anticoagulants:
  Fluoride and citrate inhibit the enzyme activity. The only accepted anticoagulants are heparin and EDTA.
- Bilirubin:
  No interference from free bilirubin up to a level of 15 mg/dl, and from conjugated bilirubin up to level of 6.8 mg/dl.
- Drugs:
  Young et al. in 1990 published a comprehensive list of drugs and substances which may interfere with this assay.
- Haemolysis:
  Erythrocyte contamination may elevate results, since CPK activities in erythrocytes are three to five times higher than those in normal sera.
- Lipemia:
  Lipemic specimens may cause high absorbance flagging. Choose diluted sample treatment for automatic rerun.

WARNING & PRECAUTION

- Vitro CPK-MB reagent is for in vitro diagnostic use only. Normal precautions exercised in handling laboratory reagents should be followed.
- Warm up working solution to the corresponding temperature before use.
- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- Valid results depend on an accurately calibrated instrument, timing, and temperature control.

PERFORMANCE CHARACTERISTICS

Imprecision
Reproducibility was determined using an internal protocol. The following results were obtained:

<table>
<thead>
<tr>
<th></th>
<th>Within Run</th>
<th>Between Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Level I</td>
<td>Level II</td>
</tr>
<tr>
<td>Number of samples</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Mean (U/l)</td>
<td>37</td>
<td>155</td>
</tr>
<tr>
<td>SD (U/l)</td>
<td>1.4</td>
<td>2.5</td>
</tr>
<tr>
<td>CV (%)</td>
<td>4.8</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Method Comparison
Comparison studies were carried out using another similar commercially available CK-MB reagent as a reference. Serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained:
- Number of sample pairs 45
- Range of sample results 5 - 226 U/l
- Mean of reference method results 44 U/l
- Mean of CK-MB results 45 U/l
- Slope 0.997
- Intercept 1.9 U/l
- Correlation coefficient 0.9995

Sensitivity
The sensitivity is defined as the lower detection limit represents the lowest measurable CPK(MB) activity that can be distinguished from zero.

When run as recommended the sensitivity of this assay is 4 U/l.

Linearity
When run as recommended, the assay is linear up to 1000 U/l.

If result exceeds 1000 U/l, specimen should be diluted 1+5 with 0.9% NaCl solution and reassayed. Multiply the result by 6.

BIBLIOGRAPHY


SYMBOL DECLARATION

ORDERING INFORMATION

<table>
<thead>
<tr>
<th>REF</th>
<th>SIZE</th>
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<tr>
<td>11401</td>
<td>4 X 5ml</td>
</tr>
<tr>
<td>11402</td>
<td>5 X 5 ml</td>
</tr>
<tr>
<td>11403</td>
<td>6 X 5 ml</td>
</tr>
<tr>
<td>11404</td>
<td>5 X 10 ml</td>
</tr>
<tr>
<td>11405</td>
<td>6 X 10 ml</td>
</tr>
<tr>
<td>11406</td>
<td>10 x 10 ml</td>
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</table>

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