The activity of the CK-B is determined using the following reaction sequence:

CK-MB + Antibody  ———— >   50% Inhibited CK-MB

During the initial incubation the following reactions take place:

CK-MB is measured which represents half the activity of CK-MB. The method assumes that the MM and one half the activity of CK-MB. The activity of the non inhibited B monomer subunit of the Thermo Scientific CK-MB method utilises an immunoinhibition method. The reagent contains that they are easily automated.

A number of methods are available for the separation and quantification of CK-MB in electrophoresis and immunoinhibition. The immunoinhibition methods have the advantage in that they are easily automated.

The Thermo Scientific CK-MB method utilises an immunoinhibition method. The reagent contains a monoclonal antibody mix to the CK-M monomer and so completely inhibits the activity of CK-MM and one half 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-NAC reagent which contains the anti M antibody. During the initial incubation the following reactions take place:

1. CK-MM + Antibody  ———— >   Inhibited CK-MM
2. CK-MB + Antibody  ———— >   50% Inhibited CK-MB
3. Inactivated CK-B  ———— >   Activated CK-B

The activity of the CK-B is determined using the following reaction sequence:

3. Creatine Phosphate + Mg-ADP  ———— >   CK-B  ———— >   Creatine + ATP
4. ATP + Glucose  ———— >   Glucose-6-phosphate + ATP
5. G6P + NADPH  ———— >   G6PDH  ———— >   6-GP + NADPH
6. 2ADP + NADPH  ———— >   AK  //  AMP + P1P5-diAP

**METHODOLOGY**

A number of methods are available for the separation and quantification of CK-MB in 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 brain and smooth muscle. CK-MB is found in a high concentration in the myocardium (between 14 and 42%) and to a lesser extent skeletal muscle. In the absence of disease, most CK activity in serum is due to the CK-MM isoform. Damage to the myocardium, as will occur in acute myocardial infarcation (AMI), will result in increased circulating levels of the CK-MB isoform. Typically CK-MB levels become elevated 4 to 6 hours after the onset of chest pain, peak between 12 to 24 hours and return to a baseline within 48 hours. Determination of CK-MB usually on admission and at 6 hours, 12 hours, and 24 hours later, is recommended when AMI is suspected.

**INTENDED USE**

This reagent is intended for the in vitro quantitative determination of CK-MB (CK-2) in human serum.

**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 brain and smooth muscle. CK-MB is found in a high concentration in the myocardium (between 14 and 42%) and to a lesser extent skeletal muscle. In the absence of disease, most CK activity in serum is due to the CK-MM isoform. Damage to the myocardium, as will occur in acute myocardial infarction (AMI), will result in increased circulating levels of the CK-MB isoform. Typically CK-MB levels become elevated 4 to 6 hours after the onset of chest pain, peak between 12 to 24 hours and return to a baseline within 48 hours. Determination of CK-MB usually on admission and at 6 hours, 12 hours, and 24 hours later, is recommended when AMI is suspected.

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In this method serum is added to a modified CK-NAC reagent which contains the anti M antibody. During the initial incubation the following reactions take place:

1. CK-MM + Antibody  ———— >   Inhibited CK-MM
2. CK-MB + Antibody  ———— >   50% Inhibited CK-MB
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4. ATP + Glucose  ———— >   Glucose-6-phosphate + ATP
5. G6P + NADPH  ———— >   G6PDH  ———— >   6-GP + NADPH
6. 2ADP + NADPH  ———— >   AK  //  AMP + P1P5-diAP

**CAPITALS IN PRODUCT LABELLING**

- **EC REP**
- **FDN REP**
- **LOT**
- **REF**

**Hazard Statements**

H360 May damage fertility or the unborn child

**Precautionary Statements - Prevention**

Obtain special instructions before use

Do not handle until all safety precautions have been read and understood

Use personal protective equipment as required

**Precautionary Statements - Response**

IF exposed or concerned: Get medical advice/attention

**Precautionary Statements - Storage**

Store locked up

**Precautionary Statements - Disposal**

Dispose of contents/container to an approved waste disposal plant

Hazardous not otherwise classified (HNOC)

Not applicable

Unknown Toxicity

0.448% of the mixture consists of ingredient(s) of unknown toxicity

Other information

No information available

Refer to the product Safety Data Sheet for additional information.

**STABILITY AND STORAGE**

Prior to use:

When stored refrigerated at 2-8°C the reagent is stable until the expiration date stated on the bottle and kit box label.

Reconstituted Reagent:

When stored capped at 2-8°C, the reagent is stable for at least 7 days.

**Additional Equipment Required But Not Provided**

- If required, pipettes for accurately dispensing measured volumes.
- A clinical chemistry analyser capable of maintaining constant temperature (37°C) and measuring absorbance at 340 nm.
- Analyser specific consumables, eg: sample cups.
- Normal and Abnormal control material.

**ASSAY PROCEDURE**

The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

**SYSTEM PARAMETERS**

- **Temperature** 30°-37°C
- **Wavelength** 340 nm (334, 365 nm)
- **Assay Type** Rate/Kinetic
- **Direction** Increase
- **Sample Reagent Ratio** 1:20
  - eg: Sample Vol 10 µL
  - Reagent Vol 200 µL
- **Delay/Lag Time** 300 seconds
- **Read Time** 300 seconds

**INTENDED USE**

This reagent is intended for the in vitro quantitative determination of CK-MB (CK-2) in human serum.
Reagent Blank  Low  0.0 AU  (340 nm, 1 cm lightpath)  High  0.7 AU  Linearity  Up to 1000 U/L  Sensitivity  0.15 μmol/min per U/L  (340 nm, 1 cm lightpath)

CALCULATIONS

Results are calculated, usually automatically by the instrument, as follows:

Activity in U/L = \( \frac{\text{Abs} \times \text{min} \times \text{Factor}}{2 \times \text{SV} \times \text{P}} \)

where:  
\( \text{SV} \) = Sample volume in mL  
\( \text{P} \) = Cuvette pathlength in cm.  
2 = Multiplication of the CK-B value by 2 gives an estimation of the CK-MB activity.

Percentage of CK-MB:

\[ \% \text{ CK-MB activity} = \left( \frac{\text{CK-MB U/L}}{\text{Total CK U/L}} \right) \times 100 \]

Example:

\[ \text{Total CK} = 350 \text{ U/L} \]
\[ \text{CK-MB} = 53 \text{ U/L} \]
\[ \% \text{ CK-MB activity} = \left( \frac{53 \text{ U/L}}{350 \text{ U/L}} \right) \times 100 = 15\% \]

NOTES

1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. The total CK should be determined first using either the Thermo Scientific IFCC single vial CK reagent or 2 vial IFCC CK reagent. If the change in absorbance is greater than 0.55/min, use the assay with diluted serum. However, the volume fraction of serum in the CK reaction system is critical. Changes in the volume fraction, as will occur in sample predilution, does not produce stoichiometric changes in the reaction rate. If dilution is necessary 150 ml/min, of NaCl is recommended. At a dilution of 1:2 an apparent increase in CK of maximally 10% may be expected.1,4 Alternatively, a CK free serum pool can be used for dilution. CK free serum can be produced by heating serum at 56°C for two hours.
3. Valid results depend on accurately calibrated instruments, timing and temperature control.
4. The millimolar absorption coefficient for NADH at 334 nm = 6.18 and at 365 nm = 3.40.
5. Unit conversion U/L to 16.67 x 10^3 = μkat/L.

CALIBRATION

Not required. The rate of reaction is converted to U/L of activity by a calculation factor. Refer to the calculation section of this package insert.

QUALITY CONTROL

To ensure adequate quality control, normal and abnormal control with assayed values should be run as unknown samples:-

- At least once per day or as established by the laboratory.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.

Control results falling above or below the upper or lower limits of the established ranges indicate the assay may be out of control. 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 Technical Services or your local distributor.

LIMITATIONS

1. Studies to determine the level of interference from haemoglobin, bilirubin and lipaemia were carried out on an automated clinical chemistry analyser. The following results were obtained:

- Haemoglobin: Haemolysed samples should be avoided to minimise interference from adenylate kinase and other reaction intermediates such as ATP and G-6-P.
- Bilirubin: No interference from bilirubin up to 340 μmol/L (20 mg/dL).
- Lipaemia: No interference from lipaemia, measured as triglycerides, up to 2.4 mmol/L (210 mg/dL).
- CK-BB: If present in the serum is a potential interfering factor in this assay system. Studies have shown that CK-BB only occurs rarely in serum.6

3. Atypical isoenzymes of CK have also been found to interfere with this assay system. One form, a complex of CK-BB and immunoglobulin G (Macro CK type 1) is more frequently found in elderly women. The presence of atypical CK's does not undermine the value to the assay system as the enzyme pattern over time shows a steady state. In suspected AMI CK-MB values will rise and return to normal levels in 48 hours.7

4. Young DS has published a comprehensive list of drugs and substances which may interfere with this assay.8

EXPECTED VALUES\(^{9,10}\)

<table>
<thead>
<tr>
<th></th>
<th>At 37°C</th>
<th>At 30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total CK</td>
<td>Males &lt;200 U/L</td>
<td>Females &lt;180 U/L</td>
</tr>
<tr>
<td></td>
<td>Males &lt;130 U/L</td>
<td>Females &lt;113 U/L</td>
</tr>
</tbody>
</table>

CK-MB

<table>
<thead>
<tr>
<th></th>
<th>At 37°C</th>
<th>At 30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;25 U/L</td>
<td>&lt;16 U/L</td>
</tr>
</tbody>
</table>

CK-MB%  A CK-MB ratio between 6 - 25% is consistent with Acute Myocardial Infarction (see Limitation 3).

The quoted values are representative of the expected range for this method and should serve as a guide only. It is recommended that each laboratory verify this range or derives a reference interval for the population that it serves.

PERFORMANCE DATA

The following performance data was obtained with the CK-MB reagent on a automated Clinical Chemistry system.

IMPRECISION

Imprecision was evaluated using two levels of commercial control and following the NCCLS EP5-T procedure.9

Within Run:

<table>
<thead>
<tr>
<th></th>
<th>LEVEL I</th>
<th>LEVEL II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of data points</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Mean (U/L)</td>
<td>37</td>
<td>156</td>
</tr>
<tr>
<td>SD (U/L)</td>
<td>1.7</td>
<td>2.5</td>
</tr>
<tr>
<td>CV (%)</td>
<td>4.6</td>
<td>1.6</td>
</tr>
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</table>

Between Day:

<table>
<thead>
<tr>
<th></th>
<th>LEVEL I</th>
<th>LEVEL II</th>
</tr>
</thead>
<tbody>
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<tr>
<td>Mean (U/L)</td>
<td>37</td>
<td>156</td>
</tr>
<tr>
<td>SD (U/L)</td>
<td>1.3</td>
<td>3.3</td>
</tr>
<tr>
<td>CV (%)</td>
<td>3.4</td>
<td>2.1</td>
</tr>
</tbody>
</table>

MEASURING RANGE

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

SPECIFICITY

Inhibition studies carried out indicate that CK-MB isoenzyme Reagent effectively inhibited greater than 99% of all CK-MM activity in a sample with 2000 U/L CK-MM.

ANALYTICAL SENSITIVITY

When run as recommended the sensitivity of the assay is 0.15 μmol/min per U/L.

REFERENCES

2. Chapman JF, Woodard LL and Silverman LM. Creatine kinase isoenzymes in Clinical Chemistry system.

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