**CREATINE KINASE -MB (CK-MB)**

**OSR61155**

<table>
<thead>
<tr>
<th></th>
<th>2 x 22 mL</th>
<th>2 x 4 mL</th>
<th>2 x 6 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1-2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Intended Use**

System reagent for the quantitative determination of Creatine Kinase-MB isoenzyme in human serum and plasma on Beckman Coulter AU analyzers.

**Summary**

Measurements of Creatine Kinase (EC 2.7.3.2) are used in the diagnosis and treatment of myocardial infarction and muscle disease, such as progressive Duchenne-type muscular dystrophy.

Creatine Kinase is a dimeric enzyme composed of M and/or B subunits which associate to form the CK-MM, CK-MB and CK-BB isoenzymes.\(^1\)\(^,\)\(^2\) Following myocardial infarction the CK-MM level rises and reaches a peak between 18-30 hrs, the increase being similar to that of the total CK activity. CK-MB also rises following MI, however, it reaches a peak up to 12 hrs earlier than CK-MM making it an important early indicator of MI. The use of Total CK and CK-MB in the diagnosis of MI is the most important single application of CK measurements in clinical chemistry.\(^3\)\(^,\)\(^4\)

**Methodology**

This CK procedure is a modification of the IFCC method.\(^4\)\(^,\)\(^5\) The R1 reagent contains an antibody which binds to the M subunit of CK in the serum sample thereby inhibiting the activity of the M subunit. The B subunit of the enzyme remains free to act on the substrate present in the R2 reagent. CK reversibly catalyzes the transfer of a phosphate group from creatine phosphate to adenosine diphosphate (ADP) to give creatine and adenosine triphosphate (ATP) as products. The ATP formed is used to produce glucose-6-phosphate and ADP from glucose. This reaction is catalyzed by hexokinase (HK) which requires magnesium ions for maximum activity. The glucose-6-phosphate is oxidized by the action of the enzyme glucose-6-phosphate dehydrogenase (G6P-DH) with simultaneous reduction of the coenzyme nicotinamide adenine dinucleotide phosphate (NADP) to give NADPH and 6-phosphogluconate. The rate of increase of absorbance at 340/660 nm due to the formation of NADPH is directly proportional to the activity of CK-MB in the sample.

\[
\text{Creatine Phosphate + ADP} \rightarrow \text{Creatine + ATP}
\]

\[
\text{ATP + Glucose} \rightarrow \text{ADP + Glucose-6-phosphate (G-6-P)}
\]

\[
\text{G-6-P + NADP}^+ \rightarrow 6\text{-phosphogluconate + NADPH + H}^+
\]

**System Information**

For AU400/400\(^e\)/480, AU600/640/640\(^e\)/680 and AU2700/5400/AU5800 Beckman Coulter Analyzers.

**Reagents**

Final concentration of reactive ingredients:

- Imidazole (pH 6.7) 100 mmol/L
- Hexokinase (HK) \(\geq 4\) KU/L (41.67 µkat/L)
- NADP 2.0 mmol/L
- G6P-DH \(\geq 2.8\) KU/L (25 µkat/L)
- ADP 2.0 mmol/L
- Mg-Acetate 10 mmol/L
- AMP 5.0 mmol/L
- Diadenosine pentaphosphate 10 µmol/L
- EDTA 2.0 mmol/L
- Glucose 20 mmol/L
- Creatine Phosphate 30 mmol/L
- N-Acetylcysteine 0.2 mmol/L
- Activator 26 mmol/L
- Antibody to CK-M subunit Variable

Also contains preservatives.

**Precautions**

1. For in vitro diagnostic use.
2. Do not ingest. Harmful if swallowed.
3. Contains sodium azide as a preservative which may react with lead joints in copper plumbing to form explosive compounds. Even though the reagent contains minute quantities of sodium azide, drains should be well flushed with water when discarding the reagent.
4. Dispose of all waste material in accordance with local guidelines.

**Preparation of Reagents**

R1: Ensure complete transfer of R1-2 into R1-1 by pouring an aliquot of R1-1 buffer into R1-2, mix gently, then transfer entire contents back into R1-1. Mix by gentle inversion before placing on board the instrument.

R2: The reagent is ready for use and can be placed directly on board the instrument. No preparation is required.
Creatine Kinase – MB (CK-MB)

Storage and Stability
1. The unopened reagents are stable until the expiration date printed on the label when stored at 2 – 8°C.
2. Opened reagents are stable for 30 days when stored in the refrigerated compartment of the analyzer.

Indications of Deterioration
Visible signs of microbial growth, gross turbidity or precipitate, or any change in reagent color may indicate degradation and warrant discontinuance of use.

Specimen Collection and Preparation
Serum and heparinized plasma samples free from hemolysis are the recommended specimens. Allow specimen to clot. Remove serum from cells promptly to minimize hemolysis and contamination by adenylyl kinase from the red cells. Plasma samples may occasionally produce unpredictable rate reactions resulting in false low results. Plasma with EDTA, oxalate or citrate is not recommended.

Sample Storage and Stability
Protect samples from light for maximum stability. CK is stable in serum for 4 hours at 15 - 25°C, 8 - 12 hours at 2 - 8°C or 1 month at ≤-20°C.

Interfering Substances
Results of studies show that the following substances interfere with this Creatine Kinase-MB assay.

The criteria for no significant interference is recovery within 10% of the initial value

<table>
<thead>
<tr>
<th>Substance</th>
<th>Interference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>No significant interference up to 40 mg/dL</td>
</tr>
<tr>
<td>Hemolysis</td>
<td>No significant interference up to 400 mg/dL</td>
</tr>
<tr>
<td>Lipemia</td>
<td>No significant interference up to 400 mg/dL</td>
</tr>
</tbody>
</table>

* Adenylate Kinase from red blood cells may react with the reagent to produce spurious results and such specimens should not be used.

** Intralipid, manufactured by KabiVitrum Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

In very rare cases gammopathy, especially monoclonal IgM (Waldenström’s macroglobulinemia), may cause unreliable results.

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc. makes no representation about the completeness or accuracy of results generated by future studies. For further information on interfering substances, refer to Young for a compilation of reported interferences with this test.

Procedure
A complete list of test parameters and operational procedure can be found in the User’s Guide appropriate to the analyzer.

Materials Provided
CK-MB Reagent.

Stability of Final Reaction Mixture
The Beckman Coulter AU analyzer automatically computes every determination at the same time interval.

Calibration
Calibration of this CK-MB procedure is based upon the theoretical extinction coefficient for NADPH, which has a molar absorptivity of 6300 at 340/660 nm.

Quality Control
During operation of the Beckman Coulter AU analyzer at least two levels of appropriate quality control material, should be tested a minimum of once a day. In addition, controls should be performed with each new lot of reagent and after specific maintenance or troubleshooting steps described in the appropriate Beckman Coulter User’s Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory’s standard procedure. Please note that recovery of non-Beckman Coulter controls may vary with reagent lots, due to changes of antisera.

Results
Results are automatically printed out for each sample in U/L at 37°C.

Dynamic Range
The CK-MB procedure is linear from 10 to 2000 U/L. Samples exceeding the upper limit of linearity should be diluted and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically utilizing the AUTO REPEAT RUN.

Note: For inhibition of adenylate kinase the recommended inhibitors AMP/Ap5A are included, but as the inhibition can never be completely 100 % a residual activity could affect low CK-MB activity results. Inhibition capacity of the anti-CK-M antibody is > 99 % at a CK-MM level of 8000 U/L. In samples where the total CK activity exceeds 8000 U/L, CK-MB should be measured using a pre-diluted sample to ensure adequate inhibition of CK-M.

Expected Values
Serum:

<table>
<thead>
<tr>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 10 U/L</td>
</tr>
</tbody>
</table>

Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Specific Performance Characteristics
The following data was obtained using the CK-MB reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained in individual laboratories may differ.
Creatine Kinase – MB (CK-MB)

**Precision**
Estimates of precision, based on CLSI recommendations, are consistent with typical performance. The within run precision is less than 5% CV or SD ≤ 2.3 U/L and total precision is less than 6.5% CV or SD ≤ 3 U/L. Assays of control sera were carried out and data reduced following CLSI guidelines above.

<table>
<thead>
<tr>
<th>N = 80</th>
<th>Within run</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean, U/L</td>
<td>SD</td>
</tr>
<tr>
<td>17</td>
<td>0.69</td>
<td>4.03</td>
</tr>
<tr>
<td>86</td>
<td>0.65</td>
<td>0.75</td>
</tr>
<tr>
<td>194</td>
<td>1.04</td>
<td>0.54</td>
</tr>
</tbody>
</table>

**Method Comparison**
Patient samples were used to compare this CK-MB Reagent on the AU640 to another Beckman Coulter AU analyzer CK-MB method (Method 2). The table below demonstrates representative performance on the AU analyzers:

<table>
<thead>
<tr>
<th>Y Method</th>
<th>AU640</th>
</tr>
</thead>
<tbody>
<tr>
<td>X Method</td>
<td>Method 2</td>
</tr>
<tr>
<td>Intercept</td>
<td>2.207</td>
</tr>
<tr>
<td>Slope</td>
<td>1.061</td>
</tr>
<tr>
<td>Correlation Coeff. (r)</td>
<td>1.000</td>
</tr>
<tr>
<td>No. of Samples (n)</td>
<td>103</td>
</tr>
<tr>
<td>Range (U/L)</td>
<td>12 - 1860</td>
</tr>
</tbody>
</table>

**Sensitivity**
Typical change in absorbance per minute for 1 U/L of CK-MB is approximately 0.12 mAbsorbance.

**Limit of Quantitation**
The Limit of Quantitation (LOQ) using serum settings for the CK-MB reagent was determined to be 4 U/L. The Limit of Quantitation (LOQ) using serum settings for the CK-MB reagent on the AU5800 was determined to be 9.42 U/L. This was determined according to CLSI protocol EP17-A and represents the lowest concentration of CK-MB that can be measured with a total imprecision of 20%.

**Note**
Macro CK is an atypical form of CK that is composed of immunoglobulin complexes of normal isoenzymes. It migrates electrophoretically between MM & MB and is found mainly in elderly women. It is of no clinical significance, but its presence may cause falsely elevated results. If Macro-CK contribution is suspected its presence should be confirmed by electrophoresis. In very rare cases gammopathy, especially monoclonal IgM (Waldenström’s macroglobulinemia) can cause unreliable results.

**References**
11. Data is on file for specific AU analyzers.

Manufactured by: Beckman Coulter, Inc., 250 S. Kraemer Blvd. Brea, CA 92821, USA