Creatine kinase is a dimeric enzyme composed of two subunits. The monomer subunit M and the monomer subunit B. These subunits combine to form 3 distinct CK isoenzymes: CK-BB (CK-1), CK-MB (CK-2) et CK-MM (CK-3).

CK-MM is the main form in skeletal muscle. CK-BB is found in brain and smooth muscle. CK-MB is found in high level in myocardium (CK-MB activity represents between 10 and 20 % total CK activity) and in lesser amount in skeletal muscle (CK-MB activity represents less than 2 % of total CK activity).

In absence of disease, most CK activity in serum is due to CK-MM. Acute myocardial infarction will result in increased CK-MB isofrom circulating in serum. This one increases between 4 and 6 hours following the beginning of the attack, then peaks between 12 and 24 hours and returns to normal within 48 hours.

** Clinica SIGNIFICANCE (1)**

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**PRINCIPLE (4) (5)**

CK-NAC modified reagent contains a polyclonal antibody (specific to the CK-M monomer) which so completely inhibits CK-MM activity and than 2 % of total CK activity).

The increase in absorbance due to the conversion of NADP+ into NADPH, measured at 340 nm, is proportional to the CK-MB activity in the specimen.

**SAFETY CAUTIONS**

BIOLABO reagents are designated for professional, in vitro diagnostic use (do not pipette with mouth).

- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

**REAGENTS PREPARATION**

Vial R1 (Enzymes-Substrate): Use a non-sharp instrument to remove aluminium cap.

REF 97217: add promptly 3 mL of vial R2 (Buffer) into the contents of vial R1.

REF 97317: add promptly the contents of vial R1 (Enzymes-Substrate), into vial R2 (Buffer).

Mix gently and wait for complete dissolution (approximately 2 minutes).

**MATERIAL REQUIRED BUT NOT PROVIDED**

1. Basic medical analysis laboratory equipment.
2. REF 95516, 95526 HDL LDL CK-MB Controls (human origin)
3. REF 95506 HDL LDLC CK-MB Calibrator (human origin)

**CALIBRATION**

Results will depend on the accuracy of the instrument calibration, the time counting, the respect of reagent/specimen ratio and the temperature control.

- Human calibrator REF 95506 HDL LDL CK-MB (calibration value determined with validated statistical techniques and metrologically controlled instrument)
- Or use the theoretical calibration factor (§ CALCULATION)
QUALITY CONTROL

- REF 95516, REF 95526 HDL LDL CK-MB Controls
- External quality control program.

It is recommended to control in the following cases:
- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:
1. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control serum and repeat the test.
3. If control is still out of range, verify analysis parameters: Wavelength, temperature, specimen/reagent ratio, time counting, calibration factor.
4. If control is still out of range, use a new vial of reagent and reassay.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

SPECIMEN COLLECTION AND HANDLING (1) (2)

Unhemolysed serum. Store at 2-8°C and away from light. Use an air-tight container to prevent the loss of CO₂.

Plasma is not recommended because anticoagulants as heparin, EDTA, citrate or fluoride interfere with the determination.

If myocardial infarction is suspected, it is recommended to collect patient after 6 hours, 12 hours and 24 hours. Minimum requested number of collects is two: 12 hours and 24 hours after symptoms appearance.

CK-MB activity in serum is stable for:
- 4 to 8 hours at room temperature.
- 1 to 2 days at 2-8°C.
- 1 month at −20°C.

INTERFERENCES (1) (3) (4) (8)

Results of tests realised on KENZA Series Analyzers are indicated at § Performances.

Haemolysis: adenylate kinase and other intermediates of the reaction as ATP (adenosine triphosphate) or G₆P (glucose-6-phosphate) interfere with the assay.

CK-BB: capable to interfere with the assay (rarely present in serum).

Atypical isoenzymes: possible interference with the assay (one form, a complex of CK-BB and immunoglobulin G, more frequently found in elderly women). The presence of atypical isoenzymes does not undermine the value of the assay as the enzymes pattern over time shows a steady state. In acute myocardial infarction, CK-MB values will raise and return to normal levels in 48 hours.

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

EXPECTED VALUES (2)

<table>
<thead>
<tr>
<th>Temperature</th>
<th>CK-MB ≤ 16 IU/L</th>
<th>CK-MB &gt; 25 IU/L</th>
</tr>
</thead>
</table>
| 30°C        | CK-MB/CK (%) CK-MB ratio between 6 and 25% is consistent with acute myocardial infarction.
| 37°C        | In case of suspicion of myocardial infarction, CK-MB values rise and return to normal levels in 48 hours.

Each laboratory should establish its own normal ranges for the population it serves.

REFERENCES

(3) YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p. 3-189 to 3-190

PERFORMANCES at 37°C ON KENZA 240TX

Linearity Range: between 11 and 800 IU/L
Detection limit: approx. 3 IU/L

Precision:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean (IU/L)</th>
<th>S.D. IU/L</th>
<th>C.V. %</th>
<th>Mean (IU/L)</th>
<th>S.D. IU/L</th>
<th>C.V. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low level</td>
<td>22.7</td>
<td>44.9</td>
<td>170</td>
<td>23.3</td>
<td>45.4</td>
<td>169.2</td>
</tr>
<tr>
<td>Normal level</td>
<td>1.0</td>
<td>1.0</td>
<td>2.6</td>
<td>1.1</td>
<td>1.3</td>
<td>3.1</td>
</tr>
<tr>
<td>High level</td>
<td>3.8</td>
<td>2.1</td>
<td>66.7</td>
<td>4.7</td>
<td>2.8</td>
<td>1.9</td>
</tr>
</tbody>
</table>

Comparison studies with commercially available reagent:
Realised on human specimens (n=100) between 10 and 750 IU/L

y = 0.9684 x + 0.4074
r = 0.9994

Interferences:

- Total bilirubin: Negative interference from 276 µmol/L
- Direct bilirubin: No interference up to 443 µmol/L
- Ascorbic acid: No interference up to 2500 mg/dL
- Glucose: No interference up to 980 mg/dL
- Turbidity: No interference of the turbidity
- Haemoglobin: Positive interference from 38 µmol/L

Other substances may interfere (see § Interferences)

On the board stability: 2 weeks

Calibration Stability: 7 days

Make a new calibration when changing reagent batch, if quality control results are found out of the established range and after maintenance operations.

MANUAL PROCEDURE

Let stand reagents and specimens at room temperature.

Pipette into a 1 cm path length thermostated cuvette:

**Reagent**

<table>
<thead>
<tr>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mL</td>
</tr>
</tbody>
</table>

**Specimen**

<table>
<thead>
<tr>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 µL</td>
</tr>
</tbody>
</table>

Mix. Read initial absorbance after 5 minutes at 340 nm and then record absorbance every minute during 5 minutes.

Calculate absorbance change per minute (ΔAbs/min).

Notes:

1. Performances and stability data’s have been validated on KENZA 240TX and KENZA 450TX.
2. With Manual Procedure on Spectrophotometer and on other automated analyzers, performances and stability should be validated by user. Applications proposal are available on request.

CALCULATION

With Serum Mutilcalibrator:

\[
\text{CK NAC Activity} = \frac{(\Delta \text{Abs/min}) \text{ Specimen}}{(\Delta \text{Abs/min}) \text{ Calibrator}} \times \text{Calibrator Activity}
\]

With Theoretical Factor:

\[
\text{Activity (U/L)} = \frac{\text{VE} \times \text{P} \times \text{X Factor}}{6.3 \times \text{VE} \times \text{P}}
\]

With Theoretical Factor:

\[
\text{Activity (U/L)} = \frac{\text{Total reacational volume (mL)}}{\text{Specimen volume (mL)}} \times \text{Molar extinction coefficient for NADPH at 340nm} \times \text{Pathlength (cm)}
\]

Example, with manual Procedure (Pathlength 1 cm, 37°C, 340 nm):

\[
\text{IU/L} = \frac{(\Delta \text{Abs/min}) \times 6667}{\mu\text{Kat/L}}
\]

Calibration factor takes into account that CK-MB activity is 2 times CK-B activity.