CK MB - Instruction for use

R1: 2 x 18 mL REF R3330000016
R2: 2 x 4.5 mL

Diagnostic reagent for quantitative in vitro determination of
creatine kinase (CK-MB) in human serum or plasma on
photometric systems

TEST PARAMETERS
Method: UV, Kinetic, Increasing Reaction,
opt. DGKC / IFCC
Wavelength: 340 nm, Hg 334 nm
Temperature: 37 °C
Sample: Serum, plasma
Linearity: up to 2000 U/L
Sensitivity: The lower limit of detection is 2 U/L.

SUMMARY [1,2]
Creatine kinase (CK) is an enzyme which consists of isoenzymes
mainly of the muscle (CK-M) and the brain (CK-B). CK exists in
serum in dimeric form as CK-MM, CK-MB, CK-BB and as
macroenzyme. Measurement of CK-MB is a specific test for
detection of cardiac muscle damage and, therefore, is used for
diagnosis and monitoring of myocardial infarction.

TEST PRINCIPLE
CK-MB consists of the subunits CK-M and CK-B. Specific antibodies
against CK-M inhibit the complete CK-MM activity (main part of
the total CK activity) and the CK-M subunit of CK-MB. Only CK-B
activity is measured, which is half of the CK-MB activity.

Creatine phosphate + ADP → creatine + ATP
Glucose + ATP → Glucose-6-Phosphate (G-6-P) + ADP
G-6-P+NADP+ → G-6-P-DH + 6-Phosphogluconolactone+NADPH+H+

REAGENT COMPOSITION

COMPONENTS
CONCENTRATION
Reagent 1
Imidazole/Good’s buffer 120 mmol/L
Glucose 25 mmol/L
N-Acetylcysteine (NAC) 25 mmol/L
Magnesium acetate 12.5 mmol/L
EDTA-Na2 2 mmol/L
NADP 2.5 mmol/L
Hexokinase (HK) ≥ 5 kU/L
Monoclonal antibodies against human CK-M; inhibiting capacity 2500 U/L
Reagent 2
Imidazole/Good’s buffer 90 mmol/L
ADP 10 mmol/L
AMP 28 mmol/L
Glucose-6-Phosphate- ≥ 15 kU/L
Dehydrogenase (G6P-DH) 50 μmol/L
Creatine phosphate 150 mmol/L

REAGENT PREPARATION

Substrate Start:
Reagents are ready to use.
Sample Start:
Mix 4 parts of Reagent 1 + 1 part of Reagent 2
(= Working Reagent)

REAGENT STABILITY AND STORAGE
Conditions: protect from light!
close immediately after use
avoid contamination
do not freeze the reagents!

Substrate Start:
Storage: at 2 – 8 °C
Stability: up to the indicated expiration date
Sample Start (Working Reagent):
Storage: at 2 – 8 °C
Stability: 2 weeks
Sample: at 15 – 25 °C
The working reagent must be protected from light!

SAMPLE STABILITY AND STORAGE
Serum, Plasma Stability ±:
at 20 – 25 °C 2 days
4 – 8 °C 7 days
at 25 °C 14 days
Discard contaminated specimens. Freeze only once!

MATERIALS REQUIRED BUT NOT PROVIDED
NoCI solution (9 g/L)
General laboratory equipment

MANUAL TEST PROCEDURE
Bring reagents and samples to room temperature.
Substrate Start

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pipette into test tubes</td>
</tr>
<tr>
<td>2</td>
<td>Sample/Calibrator</td>
</tr>
<tr>
<td>3</td>
<td>Dist. water</td>
</tr>
<tr>
<td>4</td>
<td>Reagent 1</td>
</tr>
<tr>
<td>5</td>
<td>Mix. Incubate for approximately 3 minutes. Then add:</td>
</tr>
<tr>
<td>6</td>
<td>Reagent 2</td>
</tr>
<tr>
<td>7</td>
<td>Mix. Read initial absorbance after 2 min at 37 °C and start</td>
</tr>
<tr>
<td>8</td>
<td>a stopwatch. Read absorbance again after exactly 1, 2,</td>
</tr>
<tr>
<td>9</td>
<td>3, 4 and 5 min. at 37°C</td>
</tr>
<tr>
<td>10</td>
<td>( \Delta A/min = \Delta A/min sample/calibrator - \Delta A/min blank )</td>
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</tbody>
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Sample Start

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<td>Dist. water</td>
</tr>
<tr>
<td>4</td>
<td>Working reagent</td>
</tr>
<tr>
<td>5</td>
<td>Mix. Read initial absorbance after 5 min. at 37°C and start</td>
</tr>
<tr>
<td>6</td>
<td>a stopwatch. Read absorbance again after exactly 1, 2,</td>
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CALCULATION

With factor: (light path 1 cm)
CK-MB [U/L] = \( \Delta A/min x Factor \)

Factor for 340 nm 8254
Factor for 334 nm 8414

With calibrator:
CK-MB [U/L] = \( \frac{\Delta A/min Sample}{\Delta A/min Calibrator} \times \text{Conc. Cal [U/L]} \)

UNIT CONVERSION

\( U/L \times 0.01667 = \mu\text{katal/L} \)

REFERENCE RANGES
The risk of myocardial infarction is high if the following three
conditions are fulfilled [6]:
1. CK (men) > 190 U/L (3.12 μkat/L)*
   CK (women) > 167 U/L (2.87 μkat/L)*
2. CK-MB > 24 U/L (0.40 μkat/L)*
3. CK-MB activity is between 6 and 25% of total CK activity.
   * calculated using temperature conversion factor 2.38 (25°C → 37°C)
If myocardial infarction is suspected and the conditions are not fulfilled, the infarction may be fresh. In this case the measurements should be repeated after 4 hours with fresh samples.

In healthy individuals different values are found depending on race and age [6,7]. Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary. For diagnostic purposes CK values should always be assessed in conjunction with the anamnesis, the clinical examination and other findings.

**PERFORMANCE CHARACTERISTICS**

**LINEARITY, MEASURING RANGE**
The test has been developed to determine CK-MB activities up to 2000 U/L. If that value is exceeded, samples should be diluted with NaCl solution (9 g/L) and reasayed, multiplying the result by the dilution factor.

**SENSITIVITY/LIMIT OF DETECTION**
The lower limit of detection is 2 U/L

**PRECISION (at 37 °C)**

<table>
<thead>
<tr>
<th>Intra-assay, n = 20</th>
<th>Mean [U/L]</th>
<th>SD [U/L]</th>
<th>CV [%]</th>
</tr>
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<tbody>
<tr>
<td>Sample 1</td>
<td>26.7</td>
<td>0.70</td>
<td>2.61</td>
</tr>
<tr>
<td>Sample 2</td>
<td>46.6</td>
<td>0.85</td>
<td>1.82</td>
</tr>
<tr>
<td>Sample 3</td>
<td>106</td>
<td>1.03</td>
<td>0.97</td>
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<tr>
<td>Sample 1</td>
<td>28.2</td>
<td>1.05</td>
<td>3.72</td>
</tr>
<tr>
<td>Sample 2</td>
<td>52.7</td>
<td>1.66</td>
<td>3.15</td>
</tr>
<tr>
<td>Sample 3</td>
<td>109</td>
<td>2.32</td>
<td>1.13</td>
</tr>
</tbody>
</table>

**SPECIFICITY/INTERFERENCES**

<table>
<thead>
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<th>No interference up to:</th>
<th></th>
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<tbody>
<tr>
<td>ascorbic acid</td>
<td>30 mg/dL</td>
</tr>
<tr>
<td>conjugated bilirubin</td>
<td>25 mg/dL</td>
</tr>
<tr>
<td>triglycerides</td>
<td>900 mg/dL</td>
</tr>
<tr>
<td>hemoglobin</td>
<td></td>
</tr>
</tbody>
</table>

For further information on interfering substances refer to Young DS [9].

**METHOD COMPARISON**

A comparison between this CK-MB (γ) and a commercially available test (x) using 90 samples gave following results: γ = 1.00 x + 2.08 U/L; r= 1.00.

**QUALITY CONTROL**

Control sera containing non-human CK-MB fractions are not suitable to be applied with this test due to the monoclonal antibody used in the reagent. Please take care to use controls containing exclusively human CK-MB.

**CALIBRATION**

The use of a CK-MB Calibrator is optional. Calibrators containing non-human CK-MB fractions are not suitable to be applied with this test due to the monoclonal antibody used in the reagent. Please take care to use calibrators containing exclusively human CK-MB.

**WARNINGS AND PRECAUTIONS**

1. Reagent 1 and 2: Danger
   - H360D: May damage the unborn child.
   - P201: Obtain special instructions before use.
   - P280: Wear protective gloves/protective clothing/eye protection/face protection.
   - P308+P313: If exposed or concerned: Get medical advice/attention.

2. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.

3. In very rare cases, samples of patients with gammopathy might give falsified results [10].

4. Sulfasalazine and sulfapyridine medication may lead to false results in patient samples. Blood collection must be done before drug administration.

5. Heterophile antibodies in patient samples may cause falsified results.

6. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.

7. For diagnostic purposes, the results should always be assessed with the patients’ medical history, clinical examinations and other findings.

8. For professional use only!

**WASTE MANAGEMENT**

Please refer to local legal requirements.

**REFERENCES**


