CK-NAC FS*

IFCC

Diagnostic reagent for quantitative in vitro determination of creatin kinase (CK) in serum or plasma on photometric systems

Order Information

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Kit size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 1601 99 10 021</td>
<td>R1 5 x 20 mL + R2 1 x 25 mL</td>
</tr>
<tr>
<td>1 1601 99 10 026</td>
<td>R1 5 x 80 mL + R2 1 x 100 mL</td>
</tr>
<tr>
<td>1 1601 99 10 023</td>
<td>R1 1 x 800 mL + R2 1 x 200 mL</td>
</tr>
<tr>
<td>1 1601 99 10 704</td>
<td>R1 8 x 50 mL + R2 8 x 12.5 mL</td>
</tr>
<tr>
<td>1 1601 99 10 930</td>
<td>R1 4 x 20 mL + R2 2 x 10 mL</td>
</tr>
<tr>
<td>1 1601 99 30 930</td>
<td>R1 10 x 12 mL + R2 2 x 20 mL</td>
</tr>
</tbody>
</table>

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. Elevated CK values are observed in cardiac muscle damages and in skeletal muscle diseases. Measurement of CK is used especially in conjunction with CK-MB for diagnosis and monitoring of myocardial infarction.

Method

Optimized UV-test according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) and DGKC (German Society of Clinical Chemistry).

Principle

Creatine phosphate + ADP ↔ CK Creatine + ATP

Glucose + ATP + HK ↔ Glucose-6-phosphate + ADP

Glucose-6-phosphate + NADP + G6P-DH ↔ Glucuronate-6-phosphate + NADPH + H⁺

Reagents

Components and Concentrations

| R1 | Imidazole pH 6.0 | 60 mmol/L |
|    | Glucose | 27 mmol/L |
|    | N-Acetylcysteine (NAC) | 27 mmol/L |
|    | Magnesium acetate | 14 mmol/L |
|    | EDTA-Na2 | 2 mmol/L |
|    | NADP | 2.7 mmol/L |
|    | Hexokinase (HK) | ≥ 5 kU/L |
| R2 | Imidazole pH 9.0 | 160 mmol/L |
|    | ADP | 11 mmol/L |
|    | AMP | 28 mmol/L |
|    | Diadenosine pentaphosphate | 55 µmol/L |
|    | Glucose-6-phosphate dehydrogenase (G6P-DH) | ≥ 14 kU/L |
|    | EDTA-Na2 | 2 mmol/L |
|    | Creatine phosphate | 160 mmol/L |

Storage Instructions and Reagent Stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C, protected from light and contamination is avoided. Do not freeze the reagents!

Warnings and Precautions


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

4. Reagent 2 contains animal material. Handle the product as potentially infectious according to universal precautions and good laboratory practice.

5. In very rare cases, samples of patients with gammapathy might give falsified results [9].

6. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patients’ medical history, clinical examinations and other findings.

7. For professional use only!

Waste Management

Please refer to local legal requirements.

Reagent Preparation

Substrate Start

The reagents are ready to use.

Sample start

Mix 4 parts of R1 + 1 part of R2 (e. g. 20 mL R1 + 5 mL R2) = mono reagent

Stability: 3 weeks at 2 – 8°C

2 days at 15 – 25°C

The mono reagent must be protected from light.

Materials required but not provided

NaCl solution 9 g/L and general laboratory equipment

Specimen

Serum, heparin plasma or EDTA plasma

Stability [4]: 2 days at 20 – 25°C

7 days at 4 – 8°C

4 weeks (in the dark) at –20°C

Only freeze once! Discard contaminated specimens!

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength

340 nm, Hg 365 nm, Hg 334 nm

Optical path

1 cm

Temperature

37°C

Measurement

Against reagent blank

Substrate start

Blank Sample

Sample/Calibrator - 50 µL

Dist. water 50 µL 1000 µL

Reagent 1 1000 µL 1000 µL

Reagent 2 250 µL 250 µL

Mix, read absorbance after 2 min. and start stopwatch. Read absorbance again after 1, 2 and 3 min.

ΔA/min = ΔA/min sample/calibrator

Sample start

Sample/Calibrator - 40 µL

Dist. water 40 µL 1000 µL

Mono reagent 1000 µL 1000 µL

Mix, read absorbance after 3 min. and start stopwatch. Read absorbance again after 1, 2 and 3 min.

ΔA/min = ΔA/min sample/calibrator

Calculation

With factor

From absorbance readings calculate ΔA/min and multiply by the corresponding factor from table below:

ΔA/min x factor = CK activity [U/L]

| 340 nm | 4127 |
| 334 nm | 4207 |

With calibrator

CK [U/L] = \( \frac{\Delta A}{\min \, \text{Sample}} \times \text{Conc. Calibrator [U/L]} \)

Conversion factor

CK [U/L] x 0.0167 = CK [µkat/L]
Calibrators and Controls
For the calibration of automated photometric systems, DiaSys TruCal U calibrator is recommended. This method has been standardized against the original IFCC formulation. For internal quality control DiaSys TruLab N and P controls should be assayed. Each laboratory should establish corrective action in case of deviations in control recovery.

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Kit size</th>
</tr>
</thead>
<tbody>
<tr>
<td>TruCal U</td>
<td>5 9100 99 10 006 20 x 3 mL</td>
</tr>
<tr>
<td>TruLab N</td>
<td>5 9000 99 10 006 20 x 5 mL</td>
</tr>
<tr>
<td>TruLab P</td>
<td>5 9050 99 10 006 20 x 5 mL</td>
</tr>
</tbody>
</table>

Performance Characteristics

Measuring range
On automated systems the test is suitable for the determination of CK activities up to 1100 U/L. In case of a manual procedure, the test is suitable for CK activities which correspond to a maximum of ΔA/min of 0.25 at 334 and 340 nm or 0.14 at 365 nm. If such values are exceeded the samples should be diluted 1:9 with NaCl solution (9 g/L) and results multiplied by 10.

Specificity/Interferences
No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin up to 40 mg/dL, hemoglobin up to 200 mg/dL and lipemia up to 2000 mg/dL triglycerides. For further information on interfering substances refer to Young DS [5].

Sensitivity/Limit of Detection
The lower limit of detection is 1 U/L.

<table>
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<tr>
<th>Precision</th>
<th>Intra-assay precision</th>
<th>Inter-assay precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 20</td>
<td>Mean [U/L] SD [U/L] CV [%]</td>
<td>Mean [U/L] SD [U/L] CV [%]</td>
</tr>
<tr>
<td>Sample 1</td>
<td>159 3.18 2.00</td>
<td>Sample 1</td>
</tr>
<tr>
<td>Sample 2</td>
<td>220 1.54 0.70</td>
<td>Sample 2</td>
</tr>
<tr>
<td>Sample 3</td>
<td>508 3.69 0.73</td>
<td>Sample 3</td>
</tr>
</tbody>
</table>

Method Comparison
A comparison of DiaSys CK-NAC FS (y) with the IFCC reference reagent (x) using 51 samples gave following results: y = 0.997 x – 0.249 U/L; r = 0.999

A comparison of DiaSys CK-NAC FS (y) with a commercially available test (x) using 51 samples gave following results: y = 1.03 x + 0.059 U/L; r = 1.000

Reference Range

Adults [6]
Women < 145 U/L < 2.42 µkat/L
Men < 171 U/L < 2.85 µkat/L

These reference ranges ensure high diagnostic sensitivity. The diagnostic specificity is low; however, it can be improved by additional measurement of CK-MB.

Myocardial infarction: The risk of myocardial infarction is high if following three conditions are fulfilled [7]:
1. CK (Men) > 190 U/L (3.17 µkat/L)*
2. CK (Women) > 167 U/L (2.78 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 [7,8].

Children [1]

<table>
<thead>
<tr>
<th>Newborns</th>
<th>≤ 5 days</th>
<th>&lt; 6 months</th>
<th>&gt; 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Umbilical cord blood</td>
<td>175 – 402 U/L</td>
<td>195 – 700 U/L</td>
<td>24 – 229 U/L</td>
</tr>
<tr>
<td>Adults</td>
<td>468 – 1200 U/L</td>
<td>7.80 – 20.0 µkat/L</td>
<td>0.40 – 3.82 µkat/L</td>
</tr>
</tbody>
</table>

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.

Literature

Manufacturer
DiaSys Diagnostic Systems GmbH
Alte Strasse 9 65558 Holzheim Germany