CK – NAC Fluid 5+1
IN VITRO TEST FOR THE QUANTITATIVE DETERMINATION OF TOTAL CREATINE KINASE (CK) IN HUMAN SERUM AND PLASMA.

SUMMARY
Creatine kinase (CK) is a dimeric enzyme occurring in four different forms: a mitochondrial isoenzyme and the cytosolic isoenzymes CK-MM (muscle type), CK-BB (brain type) and CK-MB (myocardial type). The determination of CK and CK-isoenzyme activities is utilized in the diagnosis and monitoring of myocardial infarction and myopathies such as the progressive Duchenne muscular dystrophy. Following injury to the myocardium, such as occurs with acute myocardial infarction, CK is released from the damaged myocardial cells. In early cases, a rise in the CK activity can be found just hours after an infarction. The CK-activity reaches a maximum after 12–24 hours and then falls back to the normal range after 3–4 days. Myocardial damage is very likely when the total CK activity is above 190 U/l, the CK-MB activity is above 24 U/l (37°C) and the CK-MB activity fraction exceeds 6% of the total.

The assay method using creatine phosphate and ADP was first described by Oliver, modified by Rosalki and further improved for optimal test conditions by Szaasz. CK is rapidly inactivated by oxidants. Standardized methods for the determination of CK using the “reverse reaction” and activation by NAC were recommended by the German Society for Clinical Chemistry (DGKC) and the International Federation of Clinical Chemistry (IFCC) in 1977 and 1990 respectively.

TEST PRINCIPLE
UV test
• Sample and addition of R1
• Addition of R2 and start of reaction:
  creatine phosphate + ADP → creatine + ATP
  glucose + ATP → glucose + 6-P + ADP
  glucose + 6-P + NADPH → gluconate + 6-P + NADP + H+
Equimolar quantities of NADPH and creatine are formed at the same rate. The photometrically measured rate of formation of NADPH is proportional to the CK activity.

WORKING SOLUTION CONCENTRATION
R1 Buffer
Imidazole buffer pH 6.7 100 mmol/l
Mg-acetat 20 mmol/l
Glucose 10 mmol/l
N-acetyl-cysteine 20 mmol/l
NADP 2 mmol/l
G6P-DH 1500 U/l
HK 2500 U/l
EDTA 2 mmol/l
R2 substrate
creatin phosphate 30 mmol/l
ADP 2 mmol/l
AMP 5 mmol/l
Adenosine 10 μmol/l

REAGENT HANDLING
R1: Ready for use
R2: Ready for use

STORAGE AND STABILITY
Unopened kit components: Up to the expiration date at 2–8°C

PRECAUTIONS AND WARNINGS
For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.

SPECIMEN COLLECTION AND PREPARATION
Collect serum using standard sampling tubes.
Heparin or EDTA plasma
Stability:
  2 days at 20–25°C
  7 days at 4–8°C
  4 weeks at −20°C

TESTING PROCEDURE

Manual procedure sample start:
Wavelength Hg 334 nm, 340 nm or Hg 365 nm
Temperature +37°C
Cuvette 1 cm light path
Zero adjustment against air
Mix R1 and R2 in ratio 5+1. This solution is stable:
  up to 20 days at +2°C to +8°C or
  up to 4 days at +18°C to +22°C
Reagent mixture
Serum / plasma
  1000 μl
  40 μl

Mix and incubate for 2 minutes at +37°C. Measure after 1, 2 and 3 minutes. Calculate the mean average value (ΔA/min) put into calculation.

Calculation:
Wavelength
  334 nm 340 nm 365 nm
F x ΔA/min (U/l)
  4207 4127 7429
F x ΔA/min (µkat/l)
  70.1 68.8 123.8

Manual procedure substrat start:
Wavelength Hg 334 nm, 340 nm or Hg 365 nm
Temperature +37°C
Cuvette 1 cm light path
Zero adjustment against air
R 1
Serum / plasma
  1000 μl
  40 μl
Mix well and start.
R 2
  200 μl

Mix well and incubate for 2 minutes at +37°C. Measure after 1, 2 and 3 minutes. Calculate the mean average value (ΔA/min) put into calculation.

Calculation:
Wavelength
  334 nm 340 nm 365 nm
F x ΔA/min (U/l)
  5016 4921 8857
F x ΔA/min (µkat/l)
  83.4 82.0 147.6
**LINEARITY**

\[ \Delta A/\text{min} \text{ (Hg 334) 0.250 / (Hg 340) 0.250 / (Hg 365) 0.140} \]

**REFERENCE RANGE**

Men: 38–174 U/l (0.65–2.96 µkat/l)

Women: 26–140 U/l (0.46–2.38 µkat/l)

Each laboratory should investigate the transferability of the expressed values to its own patient population and if necessary determine its own reference range. For diagnostic purposes, CK results should always be assessed in conjunction with the patient’s medical history, clinical examination and other findings.

**QUALITY CONTROL**

For quality control, use Precinorm / Precipath by Roche, DUOTROL standard and non-standard material.

The control intervals and limits should be adapted to the individual laboratory and country-specific requirements.

Values should fall within the established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

**LIMITATIONS - INTERFERENCE**

Criterion: Recovery within ± 10% of initial value.

- icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 60 mg/dl).
- Hemolysis: No significant interference up to an H index of 200 (approximate hemoglobin concentration: 200 mg/dl).
- Lipemia (Intralipid): No significant interference up to an L index of 1000 (approximate triglycerides concentration: 2000 mg/dl).

**IMPRECISION**

Reproducibility was determined using human samples and controls in an internal protocol (within run \( n = 21 \), between day \( n = 10 \)). The following results were obtained.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean U/l</th>
<th>SD U/l</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human serum</td>
<td>31</td>
<td>0.82</td>
<td>2.6</td>
</tr>
<tr>
<td>Precinorm U</td>
<td>224</td>
<td>0.99</td>
<td>0.4</td>
</tr>
<tr>
<td>Precipath U</td>
<td>484</td>
<td>2.19</td>
<td>0.5</td>
</tr>
</tbody>
</table>

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<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Human serum</td>
<td>150</td>
<td>3.2</td>
<td>2.1</td>
</tr>
<tr>
<td>Precinorm U</td>
<td>220</td>
<td>3.1</td>
<td>1.7</td>
</tr>
<tr>
<td>Precipath U</td>
<td>475</td>
<td>8.1</td>
<td>1.7</td>
</tr>
</tbody>
</table>

**MEASURING/REPORTABLE RANGE**

2-2300 U/l (0.03–38.41 µkat/l)

Determine samples with higher activities via the rerun function. On instruments without rerun function, manually dilute samples with 0.9% NaCl or distilled/deionised water (e.g. 1 + 10). Multiply the result by the appropriate dilution factor (e.g. 11).

**ANALYTICAL SENSITIVITY (lower detection limit):** 2 U/l (0.03 µkat/l)

The detection limit represents the lowest measurable CK activity that can be distinguished from zero. It is calculated as three standard deviations of 21 replicates of the lowest standard.

**LITERATURE**