**CK-NAC REAGENT**  
**CREATINE KINASE, ACTIVATED BY N-ACETYL CYSTEINE**

### PRODUCT SUMMARY

**Stability:** 7 days at 2-8°C  
**Linear Range:** Up to 1500 U/L  
**Specimen Type:** Serum or plasma  
**Method:** Kinetic  
**Reagent Preparation:** Add specified volume of distilled or deionized water.

### METHODOLOGY

The CK-NAC reagent is based on the method of Oliver1 modified by Rosalki2 and Szasz.3 The series of reactions involved in the assay system is as follows:

1. Inactivated CK + NAC → Reactivated CK
2. Creatine Phosphate + Mg-ADP → ATP + Creatine
3. ATP + Glucose → ADP + G-6-P
4. G-6-P + NADP⁺ → 6-PG + NADPH + H⁺
5. 2ADP + AK → AMP + ATP (Inhibited by APSA and AMP)

1. As CK in serum is rapidly inactivated, in order to ensure full catalytic activity, the CK molecule must be reactivated by a thiol compound. During the first stage, sample incubates with the thiol compound N-acetyl cysteine (NAC) which reactivates the CK molecule by rapidly reducing oxidised sulfhydryl compounds at the active site.
2. In the second stage the substrate enzyme creatine phosphate initiates a series of catalysed reactions. In the first of these reactions CK catalyses the formation of ATP from creatine phosphate and ADP.
3. ATP formed in 2 is used to form glucose-6-phosphate in a reaction catalysed by Hexokinase.
4. Glucose-6-phosphate produced in 3 is oxidised to 6-phosphogluconate and NADP is reduced to NADPH in a reaction catalysed by Glucose-6-phosphate dehydrogenase.
5. AMP and P1P5-D(adenosine-5’-)pentaphosphate (APSPA) are added to inhibit adenylate kinase (myokinase) activity.

### CLINICAL SIGNIFICANCE

Creatine kinase (CK) is a dimeric enzyme composed of two types of monomer sub-units, M (Muscular) and B (Brain) which combine to form three distinct CK isoenymes, CK-1 (BB), CK-2 (MB) and CK-3 (MM). The main proportion of total CK activity is found in the skeletal muscle and this is predominantly the CK-3 isofrom. Other tissues with relatively high levels of CK include the myocardium, of which approximately 40% is the CK-2 isofrom, gastrointestinal tract and brain where the CK-1 isofrom predominates. Damage or disease to any of these tissues such as muscular dystrophy, myocardial infarction and acute cerebro vascular accident, will result in elevated blood levels of the enzyme.

### STABILITY AND STORAGE

When stored capped at 2-8°C, the reagent is stable for at least 7 days. It is recommended that when the reagent is not in use for prolonged periods of time (eg: overnight) that the reagent be capped and stored at 2-8°C.

### REAGENT COMPOSITION

- Active Ingredient: Creatine Phosphate
- Concentration: 31.5 mmol/L
- Bis / Tri Buffer
- Creatine Phosphate
- AMP
- NADP
- EDTA
- APSA
- Mg²⁺
- ADP
- D-Glucose
- N-acetyl-cysteine
- Hexokinase (yeast)
- G-6-PDH (leuconostoc)
- pH 6.80 ± 0.1 at 20°C

### SYSTEM PARAMETERS

- Temperature: 37°C
- Primary Wavelength: 340 nm (334, 365 nm)
- Secondary Wavelength: 405 nm
- Assay Type: Rate/Kinetic
- Direction: Increase
- Sample : Reagent Ratio: 1 : 20
- eg: Sample Vol: 15 µL
- Reagent Vol: 300 µL
1. Studies to determine the level of interference from haemoglobin, bilirubin (free and conjugated), lipaemia, ascorbic acid and glucose were carried out. The following results were obtained:

**Haemoglobin:** Avoid haemolysed specimens since red cells contain reaction intermediates such as ATP and G-6-P.

**Free Bilirubin:** No interference from free bilirubin up to 500µmol/L (29mg/dL).

**Conjugated Bilirubin:** No interference from conjugated bilirubin up to 500µmol/L (29mg/dL).

**Lipaemia:** No interference from lipaemia, measured as triglycerides, up to 6mmol/L (531mg/dL).

**Ascorbic Acid:** No interference from ascorbic acid up to 2.5 mmol/L.

**Glucose:** No interference from glucose up to 25 mmol/L (450 mg/dL).

2. The temperature of the fluid used to reconstitute lyophilised control serum has been reported to affect catalytic activity.¹

3. For a more comprehensive review of factors affecting urea assays refer to the publication by Young.⁴

### EXPECTED VALUES⁵

<table>
<thead>
<tr>
<th>Condition</th>
<th>Male (µkat/L)</th>
<th>Female (µkat/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>At 37°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>≤175</td>
<td>(2.9 µkat/L)</td>
</tr>
<tr>
<td>Females</td>
<td>≤140</td>
<td>(2.3 µkat/L)</td>
</tr>
<tr>
<td>At 30°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>≤105</td>
<td>(1.8 µkat/L)</td>
</tr>
<tr>
<td>Females</td>
<td>≤80</td>
<td>(1.3 µkat/L)</td>
</tr>
</tbody>
</table>

The quoted values are representative of the expected range for this method and should serve as a guide only. It is recommended that each laboratory verify this range or derives a reference interval for the population that it serves.⁵

### PERFORMANCE DATA

The following data was obtained using the CK-NAC reagent on an automated clinical chemistry analyser.

#### Within Run:

<table>
<thead>
<tr>
<th>Level</th>
<th>Number of data points</th>
<th>Mean (µ/L)</th>
<th>SD (µ/L)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>20</td>
<td>134</td>
<td>456</td>
<td>0.34</td>
</tr>
<tr>
<td>II</td>
<td>20</td>
<td>3.40</td>
<td>4.06</td>
<td>0.89</td>
</tr>
</tbody>
</table>

#### Between Run:

<table>
<thead>
<tr>
<th>Level</th>
<th>Number of data points</th>
<th>Mean (µ/L)</th>
<th>SD (µ/L)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>20</td>
<td>132</td>
<td>428</td>
<td>0.35</td>
</tr>
<tr>
<td>II</td>
<td>20</td>
<td>3.51</td>
<td>15.24</td>
<td>0.56</td>
</tr>
</tbody>
</table>

#### ACCURACY:

Comparison studies were carried out using another similar commercially available CK-NAC reagent as a reference. Serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained:

<table>
<thead>
<tr>
<th>Number of sample pairs</th>
<th>Mean of reference method results</th>
<th>Mean of CK-NAC results</th>
<th>Slope</th>
<th>Intercept</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>141 U/L</td>
<td>140 U/L</td>
<td>0.997</td>
<td>2.5 U/L</td>
<td>0.9995</td>
</tr>
</tbody>
</table>

### LINEARITY:

When run as recommended, the assay is linear to 1500 U/L.

### SENSITIVITY:

When run as recommended the sensitivity of this assay is 0.30 µmol/min per U/L.

### REFERENCES


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