Creatine Kinase (CK) Liquid Reagent

REF: 238 001 (6 x 5 ml) 30 Test
REF: 238 002 (6 x 20 ml) 120 Test
REF: 238 004 (6 x 10 ml) 60 Test

Intended Use
Spectrum Diagnostics Creatine Kinase (CK) reagent is intended for the in-vitro quantitative, diagnostic determination of Creatine kinase in human serum on both automated and manual systems.

Background
Creatine kinase (CK) is an enzyme which is contained in heart, brain and skeletal muscles. Thus, an increase of circulating level of CK may be associated to myocardial infarction, acute cerebrovascular disease, trauma or diseases of skeletal muscles. After a myocardial infarct, CK level begins raising between 4th and 6th hour after first acute symptoms, reaching the peak between 18th and 30th hour and coming back to normal values during the 3rd day. CK is present in three different isoenzymatic forms, which could be separated by electrophoresis or column chromatography; each form is originated in different body tissues, paying off their diagnostic determinations. The formula of present reagent is based on DGKC and IFCC recommendations.

Method
According to the recommendations of the International Federation of Clinical Chemistry (IFCC).

Assay Principle
Creatine kinase (CK) catalyzes the phosphorylation of ADP, in the presence of creatine phosphate, to form ATP and creatine. The catalytic concentration is determined from the rate of NADPH formation, measured at 340 nm, by means of the hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6PDH) coupled Reactions1,2.

\[
\text{Creative phosphate} + \text{ADP} \xrightarrow{\text{CK}} \text{Creative} + \text{ATP}
\]
\[
\text{ATP} + \text{Glucose} \xrightarrow{\text{HK}} \text{HK} + \text{Glucose-6-phosphate}
\]
\[
\text{Glucose-6-phosphate} + \text{NADP}^+ \xrightarrow{\text{G6PDH}} 6\text{-Phosphogluconate} + \text{NADPH} + \text{H}^+
\]

Reagents
Reagent 1 (pH 6.7) (Buffer / Coenzyme)
Imidazole 125 mmol/L
D-Glucose 25 mmol/L
N-Acetyl-L-Cysteine 25 mmol/L
Magnesium acetate 12.5 mmol/L
NADP 2.5 mmol/L
EDTA 2 mmol/L

Reagent 2 (Enzymes)
ADP 15.2 mmol/L
AMP 25 mmol/L
P1,P5-di (adenosine-5')- penta-phosphate 103 mmol/L
Glucose-6-phosphate Dehydrogenase (G6PDH) 9 KU/L
Creatine phosphate 250 mmol/L
Hexokinase (HK) 3 KU/L

Precautions and Warnings
Do not ingest or inhale. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

Storage and Stability
The reagents are stable up to the expiration date specified when stored at 2 – 8 °C.

Reagent preparation, Storage, and Stability
REF: 238 001 add 1 ml from R2 to one bottle of R1; mix gently
REF: 238 002 add 4 ml from R2 to one bottle of R1; mix gently
REF: 238 004 add 2 ml from R2 to one bottle of R1; mix gently
Or prepare the working solution according to the number of test required by mixing 4 volumes of R1 with 1 volume of R2. Stability: 4 weeks at 2-8°C away from light sources.

Specimen Collection and Preservation
Serum free of haemolysis or heparin plasma. Stability 2 days at 20-25 °C, 7 days at 2-8°C, 4 weeks at -20°C protected from light.

System Parameters
Wavelength 340 nm (334-365 nm)
Optical path 1 cm
Assay type Kinetic
Direction Increase
Sample: Reagent Ratio 1:25
e.g.: Reagent volume 1 ml
Sample volume 40 µL
Temperature 37 °C
Equilibration Time 60 seconds
Read time 1 to 3 minutes
Zero adjustment against air
Reagent blank limits
Sensitivity 1 U/L
Linearity 2000 U/L

Procedure
1. Pipette into a thermostatized cuvette:

<table>
<thead>
<tr>
<th>Working solution</th>
<th>1 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>40 µL</td>
</tr>
</tbody>
</table>

2. Mix and incubate 60 seconds.
3. Read initial absorbance (A) of the sample, start the stopwatch
   and read absorbance at 1 minute intervals thereafter for 3 minutes.
4. Calculate the difference between absorbances and the average absorbance differences per minute (ΔA/min).

Calculation
\[
\Delta A/\text{min} \times 4127 = \text{U/L CK}
\]
Units: One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions.

The concentration is expressed in units per liter of sample (U/L).

Expected values

<table>
<thead>
<tr>
<th>Gender</th>
<th>U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>24 - 204</td>
</tr>
<tr>
<td>Women</td>
<td>24 - 173</td>
</tr>
</tbody>
</table>

Quality Control
Normal & abnormal commercial control serum of known concentrations should be analyzed with each run.
**Performance Characteristics**

**Precision**
Within run (Repeatability)

<table>
<thead>
<tr>
<th></th>
<th>Level 1</th>
<th>Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean (U/L)</td>
<td>86</td>
<td>616</td>
</tr>
<tr>
<td>CV%</td>
<td>2.8</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Run to run (Reproducibility)

<table>
<thead>
<tr>
<th></th>
<th>Level 1</th>
<th>Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean (U/L)</td>
<td>77</td>
<td>624</td>
</tr>
<tr>
<td>CV%</td>
<td>2.5</td>
<td>0.8</td>
</tr>
</tbody>
</table>

**Methods Comparison**

A comparison between Spectrum Diagnostics CK reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.983 was obtained.

**Sensitivity**

When run as recommended, the minimum detection limit of the assay is 1 U/L.

**Linearity**

The reaction is linear up to CK concentration of 2000 U/l; specimens showing higher concentration should be diluted 1+2 using physiological saline and repeat the assay (result x 3).

**Interferences:**

No interferences were observed with haemoglobin until 5 g/L, bilirubin 20 mg/dL and triglycerides 7 mmol/L. Other drugs and substances may interfere.²⁻⁴

**References**


**ORDERING INFORMATION**

<table>
<thead>
<tr>
<th>CATALOG NO.</th>
<th>QUANTITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>238 001</td>
<td>6 x 5 ml</td>
</tr>
<tr>
<td>238 002</td>
<td>6 x 20 ml</td>
</tr>
<tr>
<td>238 004</td>
<td>6 x 10 ml</td>
</tr>
</tbody>
</table>