INTENDED USE: For the quantitative determination of Creatine Kinase levels in serum or plasma.

CLINICAL SIGNIFICANCE:
Creatine Kinase (CK) is an enzyme and its activity is highest in brain, heart muscle and skeletal muscle. It plays an important role in storing energy in the tissues. Elevated serum CK activity is of diagnostic importance in Myocardial infarction and muscular dystrophy. Increased levels are also found in cerebro vascular diseases, pulmonary infarction, polymyositis, motor-neuron disorders. Elevated levels may also be due to intra muscular injections, strenuous exercise and recent surgeries.

PRINCIPLE:
Creatine Kinase catalyses the reactions between creatine phosphate and ADP with formation of creatine and ATP. The ATP formed in presence of Glucose and Hexokinase (HK) gives ADP and glucose-6-phosphate. Glucose-6-phosphate, in presence of Glucose-6-phosphate dehydrogenase (G6PDH) reacts with NADP forming 6-phosphogluconate and NADPH. The increase in absorbance due to the reduction of NADP to NADPH measured at 340 nm is proportional to the activity of CK in the sample. The presence of N-Acetylcysteine (NAC) in the reaction mixture allows the optimal activation of the enzyme.

CK
Creatine Phosphate + ADP ⟷ Creatine + ATP
HK
ATP + Glucose ⟷ ADP + Glucose-6-phosphate
G6PDH
Glucose-6-phosphate + NADP+ ⟷ 6-phosphogluconate + NADPH + H+

EXPECTED VALUES:
Males : 24-195 IU/L
Females : 24-170 IU/L

It is recommended that each laboratory establish its own normal range representing its patient population.

KIT CONTENTS:
PCK-1
1. REAGENT – A 20 ml
2. REAGENT – B 2 ml
3. REAGENT – C 18 ml

SPECIMEN:
Un-hemolysed serum

STORAGE/ STABILITY:
All the reagents are ready to use and should be stored at 2-8°C till expiry date mentioned on the labels.

REAGENT PREPARATION:
All the reagents are ready to use and are stable till the expiration date mentioned on the labels when stored at 2-8°C.

Mix 1 volume of Reagent A with 9 volumes of Reagent B according to the requirement.
0.1 ml (100µl) of Reagent A and 0.9 ml (900µl) of Reagent B are mixed for preparing 1 ml of working reagent.

WORKING REAGENT IS STABLE FOR 15 DAYS AT 2-8°C.
Reagent solution should be protected from light.

Do not freeze the reagents

TEST PROCEDURE:
Allow the working reagent to reach room temperature before use.
Perform the assay as given below:

<table>
<thead>
<tr>
<th>Working Reagent</th>
<th>Specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000µl</td>
<td>20µl</td>
</tr>
</tbody>
</table>

Mix well and aspirate. Read the Initial absorbance after 1 minute. Repeat the absorbance readings exactly after 1, 2 and 3 minutes. Calculate the mean of Δ A / min.

CALCULATIONS:
CK-NAC conc. in IU / L = Δ A / min x 8095

QUALITY CONTROL: To ensure adequate quality control, the use of commercial reference control serum is recommended with each assay batch. Use of quality control material checks both the instrument and reagent functions.

PRECISION:
Precision studies were performed with two controls using NCCLS protocol EP5-A. The results of the precision studies are shown below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Within-run</th>
<th>Between - run</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>CV%</td>
<td>Mean</td>
</tr>
<tr>
<td>Control 1</td>
<td>120</td>
<td>1.0</td>
<td>122</td>
</tr>
<tr>
<td>Control 2</td>
<td>400</td>
<td>1.23</td>
<td>395</td>
</tr>
</tbody>
</table>

LINEARITY:
The procedure is linear up to 2000 IU/L. If values exceed this limit, dilute the sample with saline and repeat the assay. Calculate the value using the proper dilution factor.

SYSTEM PARAMETERS:
Reaction type (Mode) : Kinetic
Wave length : 340nm
Flow Cell Temp. : 37°C
Sample volume : 20 µl
Reagent volume : 1000 µl
Factor : 8095
Delay time : 60 Sec
Measuring time : 180 sec
Units : IU/L
Blank : D.Water
Low normal : 24
High normal : 190
Linearity : 2000
Reaction slope : Increasing

NOTE:
1. Do not leave reagents at room temperature when not in use.
2. Use always new tips for working reagent preparation in order to avoid contamination of reagents.
3. No interference of Bilirubin upto 20 mg/dl and Hemoglobin upto 10 gm/L.

REFERENCES:
1. IFCC methods for the measurement of catalytic concentration of enzymes, part-7; IFCC method for creatine kinase, IFCC( 1989)., 1; 130 – 139

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