PARIKSHA NEOCHEM

ALPHA AMYLASE KIT
(CNPG3 Method)

INTENDED USE: For the quantitative determination of amylase levels in serum or plasma and Urine.

CLINICAL SIGNIFICANCE:
Amylase activity tests in serum and urine are mainly used in the diagnosis of diseases of the pancreas and in the investigation of pancreatic function. Amylase is found chiefly in the saliva and in pancreatic tissue. Normally, small amounts of amylase are present in the blood, but with various forms of pancreatic disturbance large amounts of amylase are secreted into the blood by the pancreas. The activity of the amylase in serum may fluctuate rapidly rising acutely during an attack and subsiding to normal levels shortly afterward. Increased levels are found associated with acute pancreatitis, pancreatic duct obstruction, intra-abdominal diseases, mumps and bacterial parotitis. A significant amount of the serum amylase is excreted in the urine, and as a result elevation of serum activity is reflected in the rise of urinary amylase activity. Urine amylase appears to be more frequently elevated, reaches higher levels, and persists for longer periods.

PRINCIPLE:
In this direct method α-amylase catalyzes the hydrolysis of 2-chloro-p-nitrophenyl-α-D-maltotrioside (CNPG3) substrate at pH 6.0 forming 2-chloro-p-nitrophenol (CNP) and free glycosides. The reaction is monitored kinetically at 405 nm by the rate of formation of the colored CNP produced, proportional to the activity of the amylase in the sample.

\[
\alpha-\text{amylase} \\
10 \text{ CNPG3} \rightarrow 9\text{CNP} + 1\text{CNPG2} + \text{G3} + \text{G}
\]

This test has been formulated according to the standardized method described by IFCC. Clin Chem Lab Med 2006; 44(9) : 1146-1155.

EXPECTED VALUES:
Serum: 25-140 IU/L
Urine : 1-17 IU/L/Hr
It is recommended that each laboratory establish its own normal range representing its patient population.

KIT CONTENTS: (PAM 1)
1. Substrate Reagent 5x5 ml

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

SPECIMEN:
1. Unhemolysed serum, urine.
2. E.D.T.A, Oxalate or Citrate inhibit amylase activity and hence cannot be used.
3. Amylase in serum is reported to be stable for one week at room temperature and for 2 months when stored at 2-8°C.

TEST PROCEDURE:
Pipette into test tubes

<table>
<thead>
<tr>
<th>Substrate Reagent</th>
<th>Serum Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000 µl</td>
<td>25 µl</td>
</tr>
</tbody>
</table>

Mix thoroughly and record the reading after 1 minute and subsequently more readings with 30 seconds interval at 405 nm. Calculate the average change in absorbance per minute (Δ Abs/min).

CALCULATIONS:
Amylase in IU/L = Δ Abs/min x 4640 (Factor)

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 Mean</th>
<th>Between-run Mean</th>
<th>Total Mean</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>62</td>
<td>64</td>
<td>126</td>
<td>2.7</td>
</tr>
<tr>
<td>Control 2</td>
<td>402</td>
<td>410</td>
<td>812</td>
<td>3.3</td>
</tr>
</tbody>
</table>

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

SYSTEM PARAMETERS:
- Reaction type (Mode) : Kinetic
- Wave length : 405 nm
- Delay time : 60 sec
- Read time : 90 sec
- Flow Cell Temp. : 37°C
- Factor : 4640
- Reagent volume : 1000 µl
- Sample volume : 25 µl
- Units : IU/L
- Blank : D. Water
- Low normal : 25
- High normal : 140
- Reaction Dir. : Increasing Linearity : 2000

NOTE:
1. Saliva and sweat contain α-Amylase. To avoid possible contamination do not pipette by mouth and avoid contact of the reagent and pipette tips with the skin.
2. The expected values of amylase are dependent on the substrate used in the formulation. Results cannot be compared with the kits based on formulations using other substrates.
3. Reagent should not be used if its absorbance exceeds 0.800 at 405 nm, against distilled water.
4. If the amylase activity is above 2000 IU/L dilute the specimen suitably with normal saline. In such case the results obtained should be multiplied by dilution factor to obtain correct amylase activity.

REFERENCES: