INTENDED USE: For the quantitative determination of Alkaline phosphatase in serum or plasma.

CLINICAL SIGNIFICANCE:
Serum ALP measurements are of particular interest in the investigation of two groups of conditions: bone disease and hepatobiliary disease. Among the bone diseases, the highest levels are found in Paget’s disease and in patients with ostogenic bone cancer, and moderately rises in osteomelacia and rickets, the latter falling to normal on treatment with vitamin D.

Physiological bone growth elevates ALP in serum of growing children and a transient elevation may be found during healing of bone fractures. Causes of decreased plasma ALP level are vitamin D deficiency and hypophosphatasia, and hereditary bone disease.

The response to the liver to any form of biliary tree obstruction is to synthesize more ALP. Intrahepatic obstruction of the bile flow by invading cancer or drugs raises serum ALP. Any drug that is hepatotoxic or induces cholestasis will greatly increase serum ALP. Over 200 drugs have been shown to increase ALP in susceptible patients.

PRINCIPLE:
Alkaline phosphatase (ALP) catalyses the hydrolysis of p-nitrophenylphosphate (p-NPP) with the formation of free p-nitrophenol and inorganic phosphate, alkaline buffer acting as a phosphate-group acceptor.

The reaction is monitored kinetically at 405 nm by the rate of formation of p-nitrophenol, proportional to the activity of ALP present in the sample.

\[
\text{ALP, Mg}^{++} + \text{p-Nitrophenylphosphate} + H_2O \rightarrow \text{p-Nitrophenol} + P_i + \text{DEA}
\]

EXPECTED VALUES:
Children: Up to 800 IU/L
Adults: Up to 280 IU/L

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

KIT CONTENTS:
R1: ALP Substrate Reagent (1 x 25 ml)

STORAGE/STABILITY:
ALP Substrate Reagent is stable at 2-8°C till the expiry mentioned on the label. Reagent is ready to use and should be protected from direct light.

TEST PROCEDURE:
Pipette into clean dry test tubes

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

Mix well and read absorbance against distilled water at 405 nm as follows:

A₀ - exactly after 30 seconds
A₁: A₂: A₃ - exactly after every 1 minute for 3 minutes. Determine the average change in absorbance per minute (ΔA/minute).

CALCULATIONS:
ALP activity in IU/L = ΔA/min x 2757

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>Within-run CV%</th>
<th>Between-run CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>171</td>
<td>163</td>
<td>334</td>
<td>2.52</td>
<td>2.40</td>
</tr>
<tr>
<td>Control 2</td>
<td>410</td>
<td>423</td>
<td>834</td>
<td>2.14</td>
<td>2.04</td>
</tr>
</tbody>
</table>

LINEARITY:
The procedure is linear up to 1000 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:

<table>
<thead>
<tr>
<th>Reaction type (Mode)</th>
<th>: Kinetic</th>
<th>Reaction Dir.</th>
<th>: Increasing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wave length</td>
<td>: 405 nm</td>
<td>Blank</td>
<td>: Distilled Water</td>
</tr>
<tr>
<td>Flow Cell Temp.</td>
<td>: 37°C</td>
<td>Delay Time</td>
<td>: 30 Sec</td>
</tr>
<tr>
<td>Kinetic interval</td>
<td>: 60 Sec</td>
<td>No. of Readings</td>
<td>: 4</td>
</tr>
<tr>
<td>Reagent volume</td>
<td>: 1000 µl</td>
<td>Sample volume</td>
<td>: 25 µl</td>
</tr>
<tr>
<td>Factor</td>
<td>: 2757</td>
<td>Units</td>
<td>: IU/L</td>
</tr>
<tr>
<td>High normal</td>
<td>: 280</td>
<td>Linearity</td>
<td>: 1000</td>
</tr>
<tr>
<td>Abs.Maxim</td>
<td>≤ 1.2 Abs.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE:
1. The ALP Substrate Reagent should not be left at room temperature or exposed to light (highly photosensitive).
2. The absorbance of ALP Substrate Reagent increases slowly on storage at 2-8°C, however this does not affect its performance. Discard the Reagent, if its absorbance against distilled water exceeds 1.20
3. If the ΔA/min is greater than 0.36, dilute the sample with normal saline in a ratio of 1:10 and multiply the result with dilution factor (10).
4. The reagent may be used in several automated analysers. Instructions are available on request.

REFERENCES:

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