INTENDED USE
Alkaline Phosphatase Kit is used for the determination of Alkaline Phosphatase Activity in serum.

PRINCIPLE OF THE TEST
Alkaline Phosphatase at an alkaline pH hydrolyses di Sodium Phenylphosphate to form phenol. The Phenol formed reacts with 4-Aminoantipyrine in the presence of Potassium Ferricyanide, as an oxidising agent, to form a red coloured complex. The intensity of the colour formed is directly proportional to the activity of Alkaline Phosphatase present in the sample.

\[
\text{Alkaline Phosphatase} + \text{Phenol} \rightarrow \text{Red Coloured Complex}
\]

CLINICAL SIGNIFICANCE
Alkaline phosphatase is produced primarily in the liver and in bone. It also is produced by the placenta of a pregnant woman and, to a lesser extent, by the intestines and kidneys. Normally, the liver produces more Alkaline phosphatase than the other organs or the bones. Some conditions can release large amounts of Alkaline phosphatase into the bloodstream. These conditions include rapid bone growth (during puberty), bone disease (ostemalacia or Paget’s disease), or damaged liver cells. An Alkaline phosphatase test measures the amount of the enzyme Alkaline phosphatase in the blood.

PRESENTATION
15 Tests
L1: Buffer Reagent 60 ml
L2: Substrate Reagent 6 ml
L3: Colour Reagent 60 ml
S: Phenol Standard (10 mg/dl) 5 ml

30 Tests
L1: Buffer Reagent 120 ml
L2: Substrate Reagent 12 ml
L3: Colour Reagent 120 ml
S: Phenol Standard (10 mg/dl) 5 ml

STORAGE / STABILITY OF THE KIT
The contents of the Alkaline Phosphatase Kit are stable at 2-8°C till the expiry date mentioned on the vial/bottle labels.

SAMPLE REQUIRED
Serum which is free from hemolysis is required.

MATERIAL REQUIRED BUT NOT PROVIDED
General laboratory instrumentation like Spectrophotometer/Analyzer, Thermostatic Cuvette holder, Cuvettes, Micropipettes, Test tubes, Waterbath, Stopwatch/Timer.

REAGENT PREPARATION
All the reagents are ready to use.

TEST PROCEDURE

<table>
<thead>
<tr>
<th>Addition of Reagent</th>
<th>B (ml)</th>
<th>S (ml)</th>
<th>C (ml)</th>
<th>T (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water</td>
<td>1.05</td>
<td>1.00</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Buffer Reagent (L1)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Substrate Reagent (L2)</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Mix well and allow to stand at 37°C for 3 minutes and add</td>
<td></td>
<td></td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.05</td>
</tr>
<tr>
<td>Phenol Standard (S)</td>
<td>-</td>
<td>0.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mix well and allow to stand at 37°C for 15 minutes and add</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colour Reagent (L3)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>0.05</td>
<td>-</td>
</tr>
</tbody>
</table>

Mix well after each addition. Measure the absorbance of the Blank (Abs.B), Standard (Abs.S), Control (Abs.C) and Test (Abs.T) against distilled water.

CALCULATIONS
Total Alkaline Phosphatase activity in K.A. Units = \( \frac{\text{Abs.T} - \text{Abs.C}}{\text{Abs.S} - \text{Abs.B}} \times 10 \)

LINEARITY
If Enzyme activity exceeds 60 K.A. Units dilute the sample with distilled water and repeat the assay. Multiply the value with the proper dilution factor.
REFERENCE RANGE
Total Alkaline Phosphatase Activity: 3.0 - 13.0 K.A. Units
It is recommended that each laboratory establish its own normal range representing its patient population.

NOTE
Alkaline Phosphatase is reported to be stable in serum for 3 days at 2-8°C. In case of multiple samples to be assayed simultaneously, only one blank and standard can be run for the entire series, however for each sample a control and a test assay has to be run additionally.

REFERENCES