**ALKALINE PHOSPHATASE KIT**

(Mod. Kind & King’s method)

For the determination of Alkaline Phosphatase activity in serum.

(For In vitro Diagnostic Use Only)

**Summary**

Alkaline Phosphatase (ALP) is an enzyme of the Hydrolase class of enzymes and acts in an alkaline medium. It is found in high concentrations in the liver, biliary tract epithelium and in the bones. Normal levels are age dependent and increase during bone development. Increased levels are associated mainly with liver and bone disease. Moderate increases are seen in Hodgkins disease and congestive heart failure.

**Principle**

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

\[
\text{di Na Phenylphosphate} \quad \xrightarrow{\text{ALP}} \quad \text{Phenol} + \quad \xrightarrow{\text{pH 10.0}} \quad \text{di Na Hydrogen Phosphate}
\]

\[
\text{Phenol} + \quad \text{Alkaline Medium} \quad \xrightarrow{4 \text{- Aminoantipyrine} K_{Fe(CN)}} \quad \text{Coloured Complex}
\]

**Normal reference values**

Total ALP Activity: 3.0 - 13.0 KA Units

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

**Contents**

- **15 Tests**
  - L1 : Buffer Reagent 60 ml 120 ml
  - L2 : Substrate Reagent 6 ml 12 ml
  - L3 : Colour Reagent 60 ml 120 ml
  - S : Phenol Standard (10 mg/dl) 5 ml 5 ml
- **30 Tests**

**Storage / stability**

Contents are stable at 2-8°C till the expiry mentioned on the labels.

**Reagent Preparation**

All reagents are ready to use.

**Sample material**

Serum. Free from hemolysis. ALP is reported to be stable in serum for 3 days at 2-8°C.

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**Procedure**

- **Wavelength / filter**: 510 nm (Hg 546 nm) / Green
- **Temperature**: 37°C
- **Light path**: 1 cm

**Assay**

Pipette into four clean dry test tubes labelled as Blank (B), Standard (S), Control (C), Test (T).

<table>
<thead>
<tr>
<th>Addition Sequence</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>1.00</td>
<td>1.00</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>
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</table>

Mix well and allow to stand at 37°C for 3 minutes and add:

<table>
<thead>
<tr>
<th>Addition</th>
<th>B (ml)</th>
<th>S (ml)</th>
<th>C (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>0.05</td>
</tr>
<tr>
<td>Phenol Standard (S)</td>
<td>-</td>
<td>0.05</td>
<td>-</td>
</tr>
</tbody>
</table>

Mix well and allow to stand at 37°C for 15 minutes and add:

<table>
<thead>
<tr>
<th>Addition</th>
<th>B (ml)</th>
<th>S (ml)</th>
<th>C (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour Reagent (L3)</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>
</tr>
</tbody>
</table>

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

**Calculations**

Total ALP activity in K.A. Units:

\[
\text{Abs. T} - \text{Abs. C} \times 10 \quad \text{Abs. S} - \text{Abs. B}
\]

**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.

**Note**

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**

Kind, P.R.H., & King, E.J. (1954) J Clin. Path. 7: 322

### System Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>Reaction</td>
<td>End Point Abs.</td>
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<tr>
<td>Wavelength</td>
<td>510 nm</td>
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<tr>
<td>Zero Setting</td>
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<tr>
<td>Incub. Temp.</td>
<td>37°C</td>
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<tr>
<td>Incub. Time</td>
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<tr>
<td>Delay Time</td>
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<tr>
<td>Read Time</td>
<td>—</td>
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<tr>
<td>No. of read.</td>
<td>—</td>
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<tr>
<td>Interval</td>
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<tr>
<td>Sample Vol.</td>
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<tr>
<td>Reagent Vol.</td>
<td>3.00 ml</td>
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<tr>
<td>Standard</td>
<td>Calculate</td>
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<tr>
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</tr>
<tr>
<td>React. Slope</td>
<td>Increasing</td>
</tr>
<tr>
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<td>60 KA Units</td>
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<tr>
<td>Units</td>
<td>KA Units</td>
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