WARNING AND PRECAUTIONS
Alkaline Phosphatase Color Developer: 0.1M Sodium Hydroxide, 0.1 M Sodium Carbonate.

DANGER: CAUSES BURNS
Alkaline Phosphatase StandardL Thymolphthalein in n-Propanol 0.5 mM/L. Equivalent to 50 U/L enzyme activity when used according to the Alkaline Phosphatase Procedure.

WARNINGS AND PRECAUTIONS:
1. For in vitro diagnostics use.
   CAUTION: In vitro diagnostic reagents may be hazardous. Handle in accordance with good laboratory procedures which dictate avoiding ingestion, and eye or skin contact.
2. Specimens should be considered infectious and handled appropriately.
3. In case of contact with Alkaline Phosphatase Color Developer, wash with copious amounts of water. Do not ingest.

STORAGE AND STABILITY
Store reagent set at 2 – 8 °C (refrigerated).

REAGENT DETERIORATION
1. The Alkaline Phosphatase Substrate should be a clear amber solution. A precipitation or blue-green color would indicate deterioration.
2. The Alkaline Color Developer should be a clear colorless solution.
3. Failure of the Alkaline Phosphatase Standard to achieve assayed values of freshly prepared control sera would indicate deterioration.

SPECIMEN COLLECTION
Unhemolyzed serum is the preferred sample. Heparinized plasma may also be used. Oxalate, fluoride and EDTA inhibit alkaline phosphatase, so are unsuitable as anticoagulants. Samples should be kept cold and assayed as soon as possible after collection. A timed routine for sample collection and analysis should be established in each laboratory because ALP levels in seum or plasma, or in reconstituted control serum, rise significantly when stored at 2 – 8 °C or at room temperature.

INTERFERING SUBSTANCES
EDTA, citrate, fluoride, and oxalate inhibit alkaline phosphatase. Young et al. gives a list of drugs and other substances, which may interfere with the determination of ALP activity.

MATERIALS REQUIRED BUT NOT PROVIDED
1. Pipetting devices
2. Test tubes/rack
3. Timer
4. Spectrophotometer with a temperature controlled cuvette
5. Heating bath/block.

MANUAL PROCEDURE
1. For each sample, dispense 0.5 ml of Alkaline Phosphatase Substrate into labeled test tubes and equilibrate to 37 °C for three (3) minutes.
2. At timed intervals, add 0.05 ml (50 µl) of each standard, control, and sample to its respective test tube. Mix gently. Use deionized water as sample for Reagent Blank.
3. Incubate for exactly ten(10) minutes at 37 °C.
4. Follow the same sequences as in Step 2 add 2.5 ml Alkaline Phosphatase Color Developer at timed intervals. Mix well.
5. Set the wavelength of the spectrophotometer at 590 nm. Zero with Reagent Blank.
6. Read and record absorbance of samples.

Note: 1. If the activity is greater than 100 IU/L repeat the assay with test specimen diluted two (2) fold with normal saline and multiply the dilutes test results by two (2).
2. The final colored product is stable for 60 minutes at controlled room temperature(15–30 °C).

CALCULATIONS
\[ \Delta \text{Abs of Unk} = \text{Value of Std. (IU/L)} = (\text{Unk. (IU/L)} - \text{Abs of Std.}) \times \text{Example : Unknown Absorbance} = 0.224 \]

Standard Absorbance = 0.313

Standard value = 50 IU/L

\[ \frac{0.224 \times 50 \text{ IU/L} = 35.7 \text{ IU/L}}{0.313} \]

PROCEDURAL LIMITATIONS
This methodology measures total alkaline phosphatase irrespective of tissue or organ of...
origin. Further tests may be necessary to assist in differential diagnosis.

QUALITY CONTROL
It is recommended that controls be included in each set of assays. Commercially available control material with established alkaline phosphatase activity may be used for quality control. The assigned value of the control material must be confirmed, by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction, or procedural errors.

EXPECTED VALUES
Adults 9 – 35 IU/L at 37 °C. Children have a higher normal value. It is strongly suggested that each laboratory establish its own normal range.

PERFORMANCE CHARACTERISTICS
1. Linearity: 100 IU/L
2. Sensitivity: Based on instrument resolution of 
   \( A = 0.001 \), the present procedure has a sensitivity of 0.16 IU/L.
3. Comparison: A study performed between the present procedure and one commercial product resulted in a coefficient of correlatin of 0.99 with a regression of \( y = 1.02x + 0.75 \).
4. Precision studies:
   
<table>
<thead>
<tr>
<th>Within Run</th>
<th>Run-to-Run</th>
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<tbody>
<tr>
<td>Mean IU/L</td>
<td>S.D.</td>
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<tr>
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<td>1.1</td>
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<tr>
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<td>84.3</td>
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REFERENCES