INTENDED USE

Vitro ALP reagent is intended for the in vitro quantitative determination of alkaline phosphatase in serum on manual systems.

BACKGROUND

Alkaline phosphatase refers to a group of phosphatases (pH optimum approximately 10) found in almost every tissue in the body. Most alkaline phosphatase in normal adult serum is from the liver or biliary tract. Normal alkaline phosphatase levels are age dependent with young children and adolescents having much higher levels than adults. Adult males tend to have higher levels than females, but pregnant females have increased levels due to placental secretion of alkaline phosphatase. Alkaline phosphatase in serum consists of four structural genotypes: the liver-bone-kidney type, the intestinal type, the placental type and the variant from germ cells. It occurs in osteoblasts, hepatocytes the kidneys, spleen, placenta, prostate, leukocytes and the small intestine. The liver-bone-kidney type is particularly important. Elevation of alkaline phosphatase levels occurs in diseases such as hepatitis, cirrhosis, malignancy, chemical toxicity, and in bone diseases such as metastatic carcinoma, rickets, Paget’s disease, and osteomalacia. Moderate increase in serum alkaline phosphatase levels have been observed in Hodgkin’s disease, congestive heart failure, ulcerative colitis, regional enteritis, and intra-abdominal bacterial infections. Alkaline phosphatase levels are normally elevated during periods of active bone growth, for example, in young children and adolescents.

ASSAY PRINCIPLE

Alkaline phosphatases catalyze the hydrolysis of a wide variety of physiologic and non-physiologic phosphoric acid esters in alkaline medium. The natural substrates for these enzymes have not yet been identified. Thus, a variety of synthetic substrates have been used in assay methods for ALP, the selection of which has been largely a matter of convenience. Kay demonstrated the presence of ALP in blood using β-glycerophosphate as the substrate. This method requires measuring the rate of phosphate liberation against the background level of endogenous phosphate. Phenylphosphate was used as a substrate by King and Armstrong, in this method, the liberated phenol has been measured in a variety of ways including the use of Folin-Ciocalteau reagent, and 4-aminoantipyrine.

The series of reactions involved in the assay system is as follows:

1. Alkaline phosphatase (ALP) hydrolyzes the phenylphosphate to phenol and phosphate in the pH 10.
2. The phenol liberated formed color complex in the presence of 4-aminoantipyrine and potassium ferricyanide.
3. The presence of sodium arsenate in the reagent 3 stops the enzymatic reaction.

The intensity of the color produced is directly proportional to the catalytic ALP activity. It is determined by measuring the increase in absorbance at 510 nm.

EXPECTED VALUES

<table>
<thead>
<tr>
<th></th>
<th>Serum</th>
<th>Children</th>
<th>10 – 20 Kind &amp; King U/dl</th>
<th>71 – 142 IU/l</th>
<th>Adults</th>
<th>3 – 13 Kind &amp; King U/dl</th>
<th>21 – 52 IU/l</th>
</tr>
</thead>
</table>

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes, the ALP results should always be assessed in conjunction with the patient’s medical history, clinical examination and other findings.

REAGENTS

- **R₁** Standard (phenol) equal to 142 U/l
- **R₂** Carbonate-bicarbonate buffer, pH 10.0
  - Disodium phenylphosphate
  - 50 mmol/l
  - 5.0 mmol/l
- **R₃** 4-aminoantipyrine
  - Sodium arsenate
  - Toxic reagent
  - Toxic by inhalation and if swallowed.
  - S 28 : after contact with skin, wash immediately with plenty of water.
  - S 45 : In case of accident or if you feel unwell, seek medical advice immediately (Show label where possible)
- **R₄** Potassium ferricyanide
  - 1.5 mmol/l

- **Reagent Preparation & Stability**
  - All reagents are ready for use and stable up to the expiry date given on label when stored at 2–8°C.

SPECIMEN

- Serum is the only accepted specimen. Avoid hemolysis

- **Specimen Preparation & Stability for serum specimen**
  - Freshly collected unhemolyzed serum specimen is the preferred specimen. Heparinized plasma may also be used. Complexing anticoagulants such as citrate, oxalate, and EDTA inhibit alkaline phosphatase so are unsuitable as anticoagulants. Specimens should be kept cold and assayed as soon as possible after collection. Alkaline phosphatase levels in serum, plasma rise significantly when stored at 2-8°C, or room temperature.

METHOD

Colorimetric method according Kind & King method[^1]

**PROCEDURE**

**Wavelength**

**Temperature** 37°C

**Zero adjustment** against reagent blank

**Specimen** Serum

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Specimen Blank</th>
<th>Specimen Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>R₁</strong></td>
<td>2.0 ml</td>
<td>2.0 ml</td>
<td>2.0 ml</td>
<td>2.0 ml</td>
</tr>
</tbody>
</table>
| Incubate for 5 minutes at 37°C
| Specimen Standard | ...... | ...... | ...... | 50 μl |
|           | ...... | 50 μl    | ...... | ...... |
| Incubate for exactly 15 minutes at 37°C
| **R₂**   | 500 μl | 500 μl | 500 μl | 500 μl |
| Mix well or preferably vortex
| Specimen H₂O | ...... | ...... | 50 μl | ...... |

Mix well, incubate in dark for 10 minutes, read the absorbance of specimen \(A_{\text{sample}}\), specimen blank \(A_{\text{blank}}\), and standard \(A_{\text{standard}}\) against blank. The color is stable for 45 minutes.

**Automated Procedure**

**User defined parameters**

**Calculation** User defined parameters are available upon request

Calculate the absorbance of specimen \(A_{\text{sample}}\) by subtracting the absorbance of specimen blank from absorbance of specimen.

Then calculate the ALP activity by using the following formulae:

\[
\text{ALP activity} = \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{standard}} - A_{\text{blank}}} \times 10000
\]
ALP activity = Absorbance of Sample \times \text{Standard value}^*

Absorbance of Standard

Standard Value*:
Kind and king unit/100 ml = 20
U/l = 142

Unit conversion
Kind & King U/dl x 7.1 = IU/l

Kind and King unit is that amount of enzyme which in the given conditions liberates 1 mg of phenol in 15 minutes at 37°C.

It is recommended that controls (normal and abnormal) be included in:
- Each set of assays, or
- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Commercially available control material with established ALP/AP values may be routinely used for quality control.

Failure to obtain the proper range of values in the assay of control material may indicate:
- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

The following corrective actions are recommended in such situations:
- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact Vitro Technical Services.

INTERFERING SUBSTANCES

- Anticoagulants:
  Complexing anticoagulants such as citrate, oxalate, and EDTA must be avoided. The only acceptable anticoagulant is heparin.
- Bilirubin:
  No significant interference from free or conjugated bilirubin up to a level of 60 mg/dl.
- Drugs:
  Young7 in 1990 has published a comprehensive list of drugs and substances which may interfere with this assay.
- Haemolysis:
  Haemoglobin levels higher than 250 mg/dl decrease the apparent ALP activity significantly.
- Lipemia:
  No significant interference.
- Others:
  Pathological high levels of albumin (7.0 g/dl) increase the apparent ALP activity significantly.

WARNING & PRECAUTION

- Vitro ALP reagent is for in vitro diagnostic use only. Normal precautions exercised in handling laboratory reagents should be followed.
- Warm up working solution to the corresponding temperature before use.
- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- Valid results depend on an accurately calibrated instrument, timing, and temperature control.
- Don't use the reagent if it is turbid.

SENSITIVITY

The sensitivity is defined as the lower detection limit represents the lowest measurable ALP activity that can be distinguished from zero.

When run as recommended, the assay is linear up to 40 Kind & King U/dl or 285 IU/l.

If result exceeds 40 Kind & King U/dl or 285 IU/l, reassay using smaller volume of specimen, such as 10\(\mu\)l instead of 50\(\mu\)l. Multiply the result by 5.

BIBLIOGRAPHY


ORDERING INFORMATION

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<thead>
<tr>
<th>REF</th>
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<tbody>
<tr>
<td>1021</td>
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