**INTENDED USE**

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

**BACKGROUND**

Alkaline phosphatase refers to a group of phosphatases (pH optimum approximately 10) found in 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.

**METHOD**

The Vitro ALP reagent is based on the recommendation of the DGKC. The series of reactions involved in the assay system is as follows:

Alkaline phosphatase (ALP) hydrolyzes the colorless p-nitrophenyl phosphate to p-nitrophenol and phosphate in the presence of magnesium ions. The product of enzyme hydrolysis p-nitrophenol has a yellow color at the pH of the reaction.

\[
p\text{-Nitrophenyl phosphate} + \text{H}_2\text{O} \rightarrow \text{ALP} \rightarrow p\text{-Nitrophenol} + \text{Phosphate}
\]

The rate of p-nitrophenol formation is directly proportional to the catalytic ALP activity. It is determined by measuring the increase in absorbance at 405 nm.

**EXPECTED VALUES**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Male</th>
<th>Female</th>
<th>Children Up12 years</th>
<th>Up to 180 U/l</th>
<th>Up to 5.0 μkat/l</th>
<th>Up to 240 U/l</th>
<th>Up to 6.0 μkat/l</th>
<th>Up to 1200 U/l</th>
<th>Up to 20.0 μkat/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>25°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30°C</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37°C</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</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**

- Diethanolamine buffer pH 9.8
- Magnesium ions 0.6 mmol/l
- p-Nitrophenyl phosphate 10 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.

**PROCEDURE**

**Manual Procedure**

- Specimen Preparation & Stability
  - Serum, or heparinized plasma
  - Freshly collected serum specimen should be kept at room temperature and assayed as soon as possible but not later than 4 hours after collection. Freezing causes a loss of activity, which is slowly recovered at room temperature 18 to 24 hours after the sera are thawed. Complexing anticoagulants such as citrate, oxalate, and EDTA must be avoided. Alkaline phosphatase levels in serum, plasma rise significantly when stored at 2-8°C or room temperature.

**Automated Procedure**

User defined parameters for different autoanalyzers are available upon request.

**Calculation**

Determine the change in absorbance per minute (ΔA/min) from the linear portion of the reaction curve and calculate the ALP activity by using the following formulae:

\[
\text{U/l} = 2757 \times \Delta A \text{ 405 nm/min}
\]

One international unit (U) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under specified conditions.

The general formula for converting ΔA/min into U/l is:

\[
\text{U/l} = \Delta A \text{min} \times TV \times 1000 \times SV \times LP
\]
It is recommended that controls (normal and abnormal) be included in:

- Total reaction volume in ml
- Sample volume in ml
- millimolar absorbptivity of p-nitrophenol.
- Cuvette path length in cm

1000 Conversion of U/ml to U/l

- millimolar absorbptivity of p-nitrophenol at 405 nm= 18.75

**Unit conversion**

Multiply the result by 1.22 if the assay performed at 25°C but is to be reported at 37°C.
Multiply the result by 1.5 if the assay performed at 25°C but is to be reported at 30°C.
Multiply the result by 1.23 if the assay performed at 30°C but is to be reported at 37°C.

**Temperature correction**

Failure to obtain the proper range of values in the assay of control material may routinely be used for quality control.

The following corrective actions are recommended in such situations:

1. Repeat the same controls.
2. If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
3. If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
4. 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:**
  - Youngs16 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.**

**PERFORMANCE CHARACTERISTICS**

Reproducibility was determined using an internal protocol. The following results were obtained:

<table>
<thead>
<tr>
<th>Within Run</th>
<th>Between Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Level I</td>
<td>Level I</td>
</tr>
<tr>
<td>Level II</td>
<td>Level II</td>
</tr>
<tr>
<td>Number of samples</td>
<td>40</td>
</tr>
<tr>
<td>Mean (U/l)</td>
<td>175</td>
</tr>
<tr>
<td>SD (U/l)</td>
<td>2.28</td>
</tr>
<tr>
<td>CV (%)</td>
<td>1.5</td>
</tr>
</tbody>
</table>

**Method Comparison**

Comparison studies were carried out using another similar commercially available method. Serum and plasma (Heparin) samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained:

- Number of samples: 45
- Range of sample results: 48 – 225 U/l
- Mean of reference method results: 100 U/l
- Mean of ALP results: 105 U/l
- Slope: 1.03
- Intercept: -0.33 (-0.006 μkat/L)
- Correlation coefficient: 0.9995

**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 sensitivity of this assay is 5 U/l or 0.08 kat/l.

**LINEARITY**

When run as recommended, the assay is linear up to 900 U/l or 15 kat/l.

If result exceeds 900 U/l or 15 kat/l, specimen should be diluted 1+5 with 0.9% NaCl solution and reassayed. Multiply the result by 6.

**BIBLIOGRAPHY**