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

CLINICAL SIGNIFICANCE
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 dependant 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. Phenyl phosphate 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. Alkaline phosphatase is determined by measuring the rate of hydrolysis of various phosphate esters. p-Nitrophenyl phosphate is one such ester that was used as a substrate by Fujita in 1939.

The NS Biotec ALP reagent is based on the recommendation of the DGKCI. The series of reactions involved in the assay system is as follows:

\[
\text{Alkaline phosphatase (ALP) + p-nitrophenyl phosphate + H}_2\text{O} \xrightarrow{\text{ALP}} \text{p-nitrophenol + Phosphate}
\]

EXPECTED VALUES

<table>
<thead>
<tr>
<th>Gender</th>
<th>Male</th>
<th>Female</th>
<th>Children</th>
<th>Up 12 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Up to 270 U/l</td>
<td>Up to 240 U/l</td>
<td>Up to 1200 U/l</td>
<td>Up to 20.0 µKat/l</td>
</tr>
<tr>
<td></td>
<td>Up to 4.5 µKat/l</td>
<td>Up to 3.7 µKat/l</td>
<td>Up to 20.0 µKat/l</td>
<td></td>
</tr>
</tbody>
</table>

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.

Calculation

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

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 $\Delta A/\text{min}$ into $U/l$ is:

$$U/l = \frac{\Delta A/\text{min} \times TV \times 1000}{\Sigma \times SV \times LP}$$

Where:
- $TV$ = Total reaction volume in ml
- $SV$ = Sample volume in ml
- $\Sigma$ = millimolar absorptivity of p-nitrophenol.
- $LP$ = Cuvette path length in cm
- $1000$ = Conversion of $U/ml$ to $U/l$

* millimolar absorptivity of p-nitrophenol at 405 nm = 18.75

**Unit conversion**
- $U/l \times 16.67 \times 10^{-3} = \mu\text{kat/l}$

**Temperature correction**

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

**LINEARITY**

When run as recommended, the assay is linear up to 900 U/l or 15 $\mu$kat/l. If result exceeds 900 U/l or 15 $\mu$kat/l, specimen should be diluted 1+5 with 0.9% NaCl solution and reassayed. Multiply the result by 6.

**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 $\mu$kat/l.

**QUALITY CONTROL**

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 NS Biotec 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:
    - Youngs 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 & PRECAUTIONS**

- **NS Biotec 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.**

**BIBLIOGRAPHY**