

Principle:

Alkaline phosphatase (ALP) catalyses in alkaline medium the transfer of the phosphate group from 4-nitrophenylphosphate to 2-amino-2-methyl-1-propanol (AMP), liberating 4-nitrophenol (4-NP). The catalytic concentration is determined from the rate of 4-nitrophenol (4-NP) formation, measured at 405nm.

**Reagent Composition:**

- | | | |
|-------------------------|-----------|--|
| 1. R1 Buffer Reagent | | |
| AMP buffer pH 10.2 | 120mmol/L | |
| Magnesium acetate | 10mmol/L | |
| 2. R2 Substrate Reagent | | |
| 4-NPP | 25 mmol/L | |

Store at +2° C to +8° C

Reagent Preparation:

Mix R1 (4 part) with R2 (1 part) to use.

Storage & Stability:

Store at 2-8° C, and keep away from light. Unopened reagent is stable until expiry date stated on the label.

Sample:

Unhemolysed serum or heparinised plasma. ALP is stable in serum or plasma for:

- 2-3 days at 2-8° C.

Procedure:

Let stand reagents and specimens at room temperature.

Reaction type	Kinetic test
Wave length	405 nm
Cuvette	1 cm light path
Delay time/ measurement time	60 sec
No. of reading	03
Sample volume	20 µl

Calculations:

Calculate the result as follows:

$$\text{ALP activity (U/L)} = (\text{Abs/min}) \times 2764$$

Expected Value:

	Men	Women
Adult :	100-320 U/L	70-260 U/L

Each lab should optimize its own normal range.

Quality Control:

The assay linear up to 800 IU/L. If Δ Abs /min > 0.100, reduce specimen volume or dilute specimen with saline solution and reassay taking into account the dilution factor to calculate the result. Use always QC sera to analyze the performance of the assay.

Reference:

1. IFCC methods for the measurement of catalytic concentration of enzymes. J. Clin. Chem. Clin Biochem. 1983; 21:731-748.