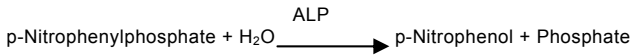




**Alkaline phosphatase
p-Nitrophenylphosphate. Kinetic. DGKC
Quantitative determination of alkaline
phosphatase (ALP)
Store at 2-8°C**

PRINCIPLE OF THE METHOD

Alkaline phosphatase (ALP) catalyses the hydrolysis of p-nitrophenyl phosphate at pH 10.4, liberating p-nitrophenol and phosphate, according to the following reaction:



The rate of p-Nitrophenol formation, measured photometrically, is proportional to the catalytic concentration of alkaline phosphatase present in the sample.

CLINICAL SIGNIFICANCE

Alkaline phosphatase is an enzyme present in almost all weaves of the organism, being particularly high in bone, liver, placenta, intestine and kidney.

Both increases and decreases of plasma ALP are clinically important. Causes of increased plasma ALP: Paget's disease of bone, obstructive liver disease, hepatitis, hepatotoxicity caused by drugs or osteomalacia. Causes of decreased plasma ALP: Cretinism and vitamin C deficiency. Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R 1 Buffer	Diethanolamine (DEA) pH 10.4 Magnesium chloride	1 mmol/L 0.5 mmol/L
R 2 Substrate	p-Nitrophenylphosphate (pNPP)	10 mmol/L

PREPARATION

Dissolve one tablet of R 2 Substrate in 15ml of R 1 Buffer. Cap and mix gently to dissolve contents. Stability: 21 days at 2-8°C or 5 days at room temperature (15-25°C).

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use. Do not use the tablets if appears broken. Do not use reagents over the expiration date.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 405 nm ≥ 1.30 .

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 405 nm.
- Thermostatic bath at 25°C / 30°C / 37°C ($\pm 0.1^\circ\text{C}$).
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

Serum or heparinized plasma. Use unhemolyzed serum, separated from the clot as soon as possible. Stability: 3 days at 2-8°C.

PROCEDURE

- 1 Assay conditions:
Wavelength: 405 nm
Cuvette : 1 cm light path
Constant temperature 25°C / 30°C / 37°C
2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a cuvette:

WR (mL)	1.2
Sample (μL)	20

4. Mix, incubate for 1 minute.
5. Read initial absorbance (A) of the sample, start the stopwatch and

read absorbances at 1 minute intervals thereafter for 3 minutes.

6. Calculate the difference between absorbances and the average absorbance differences per minute ($\Delta A/\text{min}$).

CALCULATIONS

$\Delta A/\text{min} \times 3300 = \text{U/L de ALP}$

Units: One international unit (IU) is the amount of enzyme that transforms 1 μmol of substrate per minute, in standard conditions. The concentration is expressed in units per liter of sample (U/L).

Temperature conversion factors

To correct results to other temperatures multiply by:

Assay temperature	Conversion factor to		
	25°C	30°C	37°C
25°C	1.00	1.22	2.64
30°C	0.82	1.00	1.33
37°C	0.61	0.75	1.00

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: If control values are found outside the defined range, check the instrument, reagents and technique for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES	25°C	30°C	37°C
Children (1-14 years)	< 400 U/L	< 480 U/L	< 645 U/L
Adults	60 - 170	73 - 207 U/L	98 - 279 U/L

Factors affecting ALP activities in a normal population include exercise, periods of rapid growth in children and pregnancy.

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit of 4.26 U/L to linearity limit of 825 U/L. If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

Precision

	Intra-assay (n=20)		Inter-assay (n=20)	
	Mean (U/L)	SD	CV (%)	CV (%)
Mean (U/L)	175	393	176	410
SD	2.28	5.48	4.60	10.4
CV (%)	1.30	1.40	2.61	2.53

Sensitivity: 1 U/L = 0.0003 AA/min.

Accuracy: Results obtained using ATLAS reagents (y) did not show systematic differences when compared with other commercial reagents (x). The results obtained using 50 samples were the following:

Correlation coefficient (r): 0.99.

Regression equation: $y=0.9916x - 0.4634$.

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Fluoride, oxalate, citrate and EDTA inhibit alkaline phosphate activity and should therefore not be used as anticoagulants. Haemolyses interferes due to the high concentration of alkaline phosphatase in red cells¹².

A list of drugs and other interfering substances with acid phosphatase determination has been reported by Young et al.

BIBLIOGRAPHY

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