Alkaline phosphatase

p-Nitrophenylphosphate. Kinetic. AMP buffer (IFCC)

Quantitative determination of alkaline phosphatase (ALP)

IVD

PACKAGING

Ref: 101-0592	Cont.: 10 x 10 mL

Store at 2-8º C

CLINICAL SIGNIFICANCE

Alkaline phosphatase is an enzyme present in almost all tissues of the organism, being particularly high in bone, liver, placenta, intestine and kidney. Both increases and decreases of plasma ALP are of importance clinically. 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[1,5,6]. Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE OF THE METHOD

Kinetic photometric test, according to the International Federation of Clinical Chemistry and Laboratory Medicines (IFCC).

Alkaline phosphatase (ALP) catalyses the transfer of the phosphate group from p-nitrophenylphosphate to 2-amino-2-methyl-1-propanol (AMP), liberating p-nitrophenol according to the following reaction:

p-Nitrophenylphosphate + AMP $\xrightarrow{ALP}$ p-Nitrophenol + Phosphate

The rate of p-Nitrophenol formation, measured photometrically, is proportional to the catalytic concentration of alkaline phosphatase present in the sample[1,2].

REAGENTS

R 1 Buffer	2-Amino-2-methyl-1-propanol Zinc sulfate Magnesium acetate N-hydroxyethylethylenediamine-triacetic acid (EDTA)	0.35 mol/L 1 mmol/L 2 mmol/L 2 mmol/L
R 2 Substrate	p-Nitrophenylphosphate (*p*NPP)	10 mmol/L

PREPARATION

Working reagent (WR):

Dissolve the contents of R 2 Substrate in the corresponding volume of R 1 Buffer

Stability: 21 days at 2-8º C or 5 days at room temperature (15-25º C).

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8º C, protected from light and contaminations prevented during their use. Do not freeze the reagents. 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 ≥ 1.50.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 405 nm.
- Thermostatic bath at 25º C, 30º C or 37º C (± 0.1º C)
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

Serum or heparinzed plasma[1]. Use unhemolyzed serum, separated from the clot as soon as possible. Stability: 3 days at 2-8º C.

PROCEDURE

Notes: CHRONOLAB SYSTEMS has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

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

WR (mL)	1.0
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 consecutive absorbances and the average absorbance differences per minute (ΔA/min).

CALCULATIONS

ΔA/min x 2764 = ALP ACTIVITY (U/L)

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 litre of sample (U/L).

Temperature conversion factors

To correct results to other temperatures multiply by:

Assay temperature	Conversion factor to		
	25º C	30º C	37º C
25º C	1.00	1.22	1.64
30º C	0.82	1.00	1.33
37º C	0.61	0.75	1.00

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: Contro-N (Ref. 101-0083, 101-0252) and Contro-P (Ref. 101-0084, 101-0253).

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[1]

	25º C	30º C	37º C
Adults	17 - 77 U/L	21 - 94 U/L	26 - 117 U/L

Factors affecting ALP activities in a normal population include exercise, periods of repaid 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 1.307 U/L to linearity limit of 1400 U/L. If the results obtained were greater than linearity limit, dilute the sample 1/10 with NaCl (9 g/L) and multiply the result by 10.

Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (U/L)	73	194	78	209
SD	1.67	3.03	2.13	4.90
CV (%)	2.27	1.58	2.72	2.34

Sensitivity: 1 U/L = 0.0004 ΔA / min.

Accuracy: Results obtained using CHRONOLAB 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): 0.98929.

Regression equation: y= 2.214x + 2.131.

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 cells[1,2].

A list of drugs and other interfering substances with acid phosphatase determination has been reported by Young et. al[3,4].

BIBLIOGRAPHY

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