ALKALINE PHOSPHATASE (PNPP/AMP Method)

For photometric determination of Alkaline Phosphatase (ALP) according to the recommendation of German of Clinical Chemistry .

Reagent

Reagent R1: Buffer Reagent
Reagent R2: Enzyme Reagent

Principle

ALP
p-Nirophenylphosphate + H2OPhosphate ⟶ + p- Nitrophenol

The increase in absorbance due to formation of4- nitrophenolate is measured photometrically and is directly proportional to ALP activity in sample.

Summary

Alkaline phosphatase (ALP), a hydrolytic enzyme acting optimally at alkaline pH, exists in blood in numerous distinct forms which originate mainly from bone and liver, but also from other tissues as kidney, placenta, testes, thymus, lung and tumors. Physiological increases are found during bone growth in childhood and in pregnancy, while pathological increases are largely associated with hepatobiliary and bone diseases. In hepatobiliary disease they indicate obstruction of the bile ducts as in cholestasis caused by gall stones, tumors or inflammation. Elevated activities are also observed in infectious hepatitis. In bone diseases elevated AP activities originate from increased osteoblastic activity as in Paget's disease, osteomalacia (rickets), bone metastases and hyperparathyroidism.

Storage instruction and reagent stability

The reagent is stable up to the end of the indicated monthof expiry, if stored at 2°C–8°C, protected from direct light andcontamination is avoided. Do not freeze the reagent.

Components and Concentrations

Reagent: 2-Amino methyl propanol-36.5ml/L, Zinc sulphate- 0.5gm/L, Magnesium acetae - 0.5 gm/L, p-Nitrophenylphoshate -6gm/L

Waste Management

Please refer to local, national / internationalregulatory requirements.

Reagent Preparation

Mix, 4 parts of reagent 1 and 1 part of reagent 2 = working reagent.
The stability of the working reagent is
5 days at 15°-25°C.
4 weeks at 2°- 8°C.
Reagent2 Protect the reaction solution from light.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen

Serum, Heparin plasma / EDTA Plasma
Stability: Serum @ 2°– 8°C: 1 month
@ -20°C: 3 months
Only freeze once!
Discard contaminated specimens.

Assay Procedure

Wavelength : 405nm
Temperature : 37°C

	Sample
Sample	20 µL
Reagent	1000 µL
Mix , incubate for 1 min. and read absorbance after every 60sec. for 120 sec. at 37°C.	

Calculation

Note: ΔA/min and multiply by the corresponding factor from table below:
ALP activity U/L = ΔA/min x factor.(2764)

Quality Control

For internal quality, normal and abnormal controls of Human Matrix should be assayed with each batch of samples.

Each laboratory should establish corrective action in case of deviations in control recovery.

Warnings and Precautions

1. **The standard contains animal materials. Handle the products as potentially infections according to universal precautions and good clinical laboratory practices.**
2. **For diagnostic purposes, the results should always be assessed with the patient`s medical history, clinical & other findings.**
3. **Avoid direct contact with skin and do not swallow.**
4. **In very rare cases, samples of patients with gammopathy might give falsified results.**
5. **For Professional use only!**

Performance Characteristics

Measuring Range

This test has been developed to determine Alkaline phosphatase concentrations within a measuring range from 3–1200U/L. When values exceed this range, samples should be diluted 1+1 with Na Cl solution (9 g/L) and the obtained result to be multiplied by 2.

Linearity/Limit of Maximum Detection

The higher limit of detection is 1200 U/L

Sensitivity/Limit of Detection

The lower limit of detection is 3 U/L.

Specificity / Interferences

No interference was observed by As corbic acid up to 30mg/dL, Bilirubin up to 40mg/dL and Triglycerides up to2000mg/dL.

Precision

Intra-assay n=20	Mean (mg/dL)	SD (mg/dL)	CV (%)
Sample 1	55.98	0.68	1.21
Sample 2	136.13	0.96	0.71
Sample 3	257.83	1.53	0.59

Inter-assay n=20	Mean (mg/dL)	SD (mg/dL)	CV (%)
Sample 1	76.61	0.74	0.97
Sample 2	155.94	1.36	0.87
Sample 3	225.69	1.15	0.51

Method Comparison

A comparison of Precision Biomed Alkaline Phosphatase with commercially available assay (x) using 15 samples gave following results: y = y = 0.997x – 0.740; r^2 = 0.997.

Reference Range

Adults		30°C	37°C
Women 20-25 years	(U/L)	28 - 78	42 - 98
Men 20-50 years	(U/L)	38 - 94	53 - 128
Women > 60 years	(U/L)	40 - 111	50 - 141
Men > 60 years	(U/L)	43 - 88	56 - 119

Children	(37°C)	Female	Male
1 - 30 days	(U/L)	48 - 406	75 - 319
1 Month - 1 year	(U/L)	124 - 341	82 - 383
1 - 3 years	(U/L)	108 - 317	104 - 345
4 - 6 years	(U/L)	96 - 297	093 - 309
7 - 9 years	(U/L)	69 - 325	86 - 315
10 - 12 years	(U/L)	51 - 332	42 - 362
13 - 15 years	(U/L)	50 - 162	74 - 390
16 - 18 years	(U/L)	47 - 119	52 - 171

Note:It is recommended that each laboratory should establish its own reference range based on the patient population.

Quick Reference

Parameter	Alkaline Phosphatase
Mode	Kinetic
Reaction slope	Increasing
Wavelength	405 nm
Path length	10 mm
Temperature	37°C
Reagent	1000 µL
Sample	20 µL
Delay Time	60 sec.
Read Time	60 sec.
No .of Readings	2
Factor	2764
Linearity	1200 U/L

Pack Size

Cat No.	Configuration	Pack
ALP00100	Reagent R1 - 2 x 40mL Reagent R2 - 2 x 10mL	100mL
ALP01000	Reagent R1 -2 x 400mL Reagent R2 - 2 x 100mL	1000mL

Literature

1. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007;45(9):1240–1243
2. Johnson AM, Rohlfs EM, Silverman LM. Proteins InBurtis CA, Ashwood ER. Tietz textbook of clinical chemistry. 3rd ed. Philadelphia: W. B. Saunders Company; 1999. p.477-540.
3. Thomas L. Clinical Laboratory Diagnostics. 1st ed.Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 652-6.
4. Guder WG, Zawta B et al. The Quality of DiagnosticSamples. 1st ed. Darmstadt: GIT Verlag; 2001; p. 14-5.
5. Dati F, Schumann G, Thomas L, Aguzzi F, BaudnerS,Bienvenu J et al. Consensus of a group of professional societies and diagnostic companies on guidelines for Interim reference ranges for 14 proteins in serum based on the standardization against the IFCC/BCR/CAP reference material (CRM 470). Eur J Clin Chem Clin Biochem1996;34:517-20.
6. Young DS. Effects of Drugs on Clinical LaboratoryTests.5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.

Version : ALP/00

IVD In Vitro Diagnostic Use | See Pack Insert For Procedure | Single Use only | CE

Temperature Limit | Manufacturer's Address | Manufacturing Date | Expiry Date | LOT Lot Number

Manufactured in India by :

Precision Biomed Pvt. Ltd.

Plot No. – 193, "Silver Soil Industrial Park", Village – Anantpura- Chimanpura,
Teh.- Chomu, District- Jaipur – 303702 (Rajasthan) India.
Ph. : +91-9982806050 Customer Care : +91-7820806050
E-mail : info@precisionbiomed.in, Website : www.precisionbiomed.in
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