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Alkaline Phosphatase (IFCC method)

IVD

Order Information

Cat. No.	Kit Configuration
OMR1014	Reagent 1: 2 x 40 mL
	Reagent 2: 2 x 10 mL

Summary

Alkaline Phosphatase (ALP) is hydrolytic enzyme acting ideally at alkaline pH, exists in blood in various different structures which originate from the most part from bone and liver, and also from different tissues such as kidney, placenta, testicles, thymus, lung and tumors. Physiological increments are found amid bone development in adolescence and in pregnancy, while pathological increments are to a great extent connected with hepatobiliary and bone diseases. In hepatobiliary disease they show hindrance of the bile conduits as in cholestasis brought about by gall stones, tumors or inflammation. Elevated activities of Alkaline Phosphatase are also observed in infectious hepatitis. In bone diseases elevated ALP activities start from increased osteoblastic activity as in Paget's disease, osteomalacia (rickets), bone metastases and hyperparathyroidism.

Method

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

Principle

The increase in absorbance due to formation of 4-nitrophenolate is measured photometrically and is directly proportional to ALP activity in sample.

$$\text{p-Nitrophenylphosphate} + H_2O \xrightarrow{\text{ALP}} \text{Phosphate} + \text{p-Nitrophenol}$$

Reagents Storage Instructions and Stability

Reagent are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C, protected from light and contamination is avoided. Do not freeze the reagents!
Reagent 1: Buffer
Solution Reagent 2:
Substrate Solution

Composition:

Reagent: 2-Amino methyl propanol 63 mL/L, Zinc sulphate 1 g/L, Magnesium acetate 1 g/L, p-Nitrophenylphosphate 10 g/L

Warnings and Precautions

1. Do not swallow! Avoid contact with skin and mucous membranes.
2. In very rare cases, samples of patients with gammopathy might give falsified results.
3. N-acetyl cysteine (NAC), acetaminophen and metamizole medication leads to falsely low results in patient samples.
4. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
5. For professional use only!

Waste Management

Please refer to local legal requirements.

Reagent Preparation

For Working Reagent: Mix 4 parts of Reagent 1 + 1 part of Reagent 2.
(E.g. 20 mL R1 + 5 mL R2) = Working reagent Stability: 4 weeks at 2 – 8 °C.
The Working reagent must be protected from light.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen

Serum, heparin plasma or EDTA plasma
Stability: 1 month at 2° – 8 °C, 3 months at -20 °C
Only freeze once!
Discard contaminated specimens.

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength	405nm (400-420 nm)
Optical path	1 cm
Temperature	37°C

Sample start

	Sample
Sample	20 µL
Working Reagent	1000 µL
Mix, Incubate for 1 min. and read absorbance after every 1 min. for 3 min.	

Substrate start

	Sample/Calibrator
Sample	20 µL
Reagent 1	1000 µL
Reagent 2	250 µL
Mix, Incubate for 1 min. and read absorbance after every 1 min.	

Calculation

For activity, take ΔA/min and multiply by the corresponding factor from table below:
ALP activity U/L = ΔA/min x factor.

Factor		
Sample start	405 nm	2757
Substrate start	405 nm	3433

Conversion factor

ALP [U/L] x 0.0167 = ALP [µkat/L]

Quality Controls

For internal quality control any normal and abnormal controls should be assayed with each batch of samples. Each laboratory should establish corrective action in case of deviations in control recovery.

Performance Characteristics

Measuring range
The test has been developed to determine Alkaline Phosphatase activities within a measuring range from 3-1000

U/L. When values exceed this range samples should be diluted 1 + 4 with NaCl solution (9 g/L) and the result multiplied by 5.

Specificity/Interferences

No interference was observed by, Ascorbic Acid up to 30 mg/dL, Bilirubin up to 40 mg/dL and triglycerides up to 2000 mg/dL.

Sensitivity/Limit of Detection

The lower limit of detection is 3 U/L.

Linearity

The higher limit of detection is 1000 U/L.

Precision

Intra-assay n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	267.06	4.63	1.74
Sample 2	67.63	1.21	1.79

Inter-assay n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	277.34	3.84	1.39
Sample 2	68.40	1.02	1.49

Method Comparison

A comparison of Nucleus Diagnosys Alkaline Phosphatase (y) with a commercially available test (x) using 15 samples gave following results:
$y = 0.998x + 0.128$; $r^2 = 0.997$.

Literature

1. **Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p.131-7.**

Reference Range

Adults		
Women	35-104 (U/L)	0.58 – 1.74 (µkat/L)
Men	40-129 (U/L)	0.67 – 2.15 (µkat/L)

	Male		Female	
	(U/L)	(µkat/L)	(U/L)	(µkat/L)
1 – 30 days	75 – 316	1.25 – 5.27	48 – 406	0.80 – 6.77
30 – 365 days	82 – 383	1.37 – 6.38	124 – 341	2.07 – 5.68
1 – 3 years	104 – 345	1.73 – 5.75	108 – 317	1.80 – 5.28
4 – 6 years	93 – 309	1.55 – 5.15	96 – 297	1.60 – 4.95
7 – 9 years	86 – 315	1.43 – 5.25	69 – 325	1.15 – 5.42
10 – 12 years	42 – 362	0.70 – 6.03	51 – 332	0.85 – 5.53
13 – 15 years	74 – 390	1.23 - 6.50	50 – 162	0.83 – 2.70
16-18 years	52 - 171	0.87 – 2.85	47 – 119	0.78 – 1.98

2. **Deutsche Gesellschaft fürklinische Chemie. Empfehlungen der deutschen Gesellschaft für Klinische Chemie (DGKC). Standardisierung von Methodenzur Bestimmung von Enzymaktivitätenin biologischenFlüssigkeiten. (Recommendation of the German Society of Clinical Chemistry. Standardization of methods for measurement of enzymatic activities in biological fluids.) Z Klin Chem KlinBiochem1972; 10:182-92.**
3. **Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1 st ed. Darmstadt: GIT Verlag; 2001; p. 14-5.**
4. **Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.**
5. **Fischbach F, Zawta B. Age-dependent reference limits of several enzymes in plasma at different measuring temperatures. Klin Lab 1992; 38:555-61.**
6. **Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed2007; 45(9):1240–1243.**

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