ALKALINE PHOSPHATASE (ALP) - AMP

COD 12518 5 x 20 mL
Only for *in vitro* use in the clinical laboratory

Automated Systems

BioSystems

ALKALINE PHOSPHATASE (ALP) - AMP

2-AMINO-2-METHYL-1-PROPANOL BUFFER (IFCC)

INTENDED USE

Reagent for the measurement of alkaline phosphatase (ALP)-AMP concentration in human serum or plasma. The obtained values are useful as an aid in the diagnosis and treatment of hepatobiliary and bone diseases with impaired osteoblastic activity diseases.

This reagent is for use in the BioSystems A25 and A15 analyzers or in other analyzer with similar performance characteristics.

CLINICAL SIGNIFICANCE

Alkaline phosphatase catalyzes the hydrolysis of organic phosphate monoesters at alkaline pH. The enzyme is present in practically all tissues of the body, especially at or in the cell membranes, and it occurs at particularly high concentrations in placenta, intestinal epithelium, kidney tubules, osteoblasts and liver.

The form present in the sera of normal adults originates mainly in the liver and bone.

Elevated serum ALP is found in patients with bone disease associated with increased osteoblastic activity (Paget's disease, primary and secondary hyperparathyroidism, bone tumors, rickets, osteomalacia, bone fractures) and also in patients with hepatobiliary disease (obstructive jaundice, hepatitis, hepatotoxicity caused by drugs, liver cancer). Physiological changes, such as bone growth and pregnancy, may cause increases in ALP levels[1,2].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

PRINCIPLE OF THE METHOD

Alkaline phosphatase (ALP) catalyzes in alkaline medium the transfer of the phosphate group from 4-nitrophenylphosphate to 2-amino-2-methyl-1-propanol (AMP), liberating 4-nitrophenol. The catalytic concentration is determined from the rate of 4-nitrophenol formation, measured at 405 nm[3].

$$\text{4 – Nitrophenylphosphate + AMP} \xrightarrow{\text{ALP}} \text{AMP – phosphate + 4 - Nitrophenol}$$

CONTENTS AND COMPOSITION

A. Reagent: 5 x 16 mL. 2-Amino-2-methyl-1-propanol 0.4 mol/L, zinc sulfate 1.2 mmol/L, N-hydroxyethylethylenediaminetriacetic acid 2.5 mmol/L, magnesium acetate 2.5 mmol/L, pH 10.4.

B. Reagent: For 2 x 10 mL. 4-Nitrophenylphosphate 60 mmol/L.

STORAGE AND STABILITY

Store at 2-8 ºC.

Components are stable once opened until the expiry date marked in the label if they are stored well closed and care is taken to prevent contamination during their use.

On board stability: Reagents open and kept in the refrigerated compartment of the analyzer are stable 20 days.

Indications of deterioration: Absorbance of the blank over the limit indicated in "Test Parameters".

ADDITIONAL MATERIALS REQUIRED (NOT PROVIDED)

Biochemistry Calibrator (BioSystems cod. 18011) or Biochemistry Calibrator Human (BioSystems cod. 18044).

REAGENT PREPARATION

Reagents are provided ready to use.

Working Reagent: Add 4.0 mL of the Reagent B into the Reagent A bottle. Mix gently. Other volumes can be prepared in the proportion: 4 mL Reagent A + 1 mL Reagent B.

SAMPLES

Serum and plasma collected by standard procedures.

Alkaline phosphatase in serum or plasma is stable for 7 days at 2-8ºC. Heparin may be used as anticoagulant.

CALIBRATION

A reagent blank should be done every day and a calibration at least every 20 days, after reagent lot change or as required by quality control procedures.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007. 18010 and 18043) to verify the accuracy of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if control results are not within the acceptable limits.

REFERENCE VALUES

Reaction temperature	men	women
25ºC, up to	75 U/L = 1.25 µKat/L	68 U/L = 1.13 µKat/L
30ºC, up to[4]	87 U/L = 1.45 µKat/L	80 U/L = 1.33 µKat/L
37ºC, up to[4]	115 U/L = 1.92 µKat/L	105 U/L = 1.75 µKat/L

Values at 25ºC are obtained from those at 30ºC by using a conversion factor. Concentrations in growing children are higher and highly variable.

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

METROLOGICAL CHARACTERISTICS

The metrological characteristics described below have been obtained using an A25 analyzer. Results are similar with A15.

- Detection limit: 6.0 U/L = 0.10 µkat/L.
- Linearity limit: 1200 U/L = 20 µkat/L.
- Precision:

Mean concentration	Repeatability (CV)	Within-laboratory (CV)
131 U/L = 2.18 µkat/L	4.6 %	8.9 %
318 U/L = 5.30 µkat/L	1.2 %	2.7 %

- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

LIMITATIONS OF THE PROCEDURE

- Interferences: bilirubin (up to 30 mg/dL), hemolysis (hemoglobin up to 500 mg/dL) and lipemia (triglycerides up to 1625 mg/dL) do not interfere. Other drugs and substances may interfere[5].

BIBLIOGRAPHY

1. Friedman and Young. Effects of disease on clinical laboratory tests, 4th ed. AACC Press, 2001.
2. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.
3. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37°C. Part 9. Reference procedure for the measurement of catalytic concentration of alkaline phosphatase. *Clin Chem Lab Med* 2011; 49:1439-1446.
4. Rosalki SB, Foo AY, Burlina A, at al. Multicenter evaluation of iso-ALP test kit for measurement of bone alkaline phosphatase activity in serum and plasma. *Clin Chem* 1993; 39:648-652.
5. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.

TEST PARAMETERS

These reagents may be used in several automatic analyzers. Specific instructions for application in many of them are available on request.

R1: use Reagent A, R2: use Reagent B.

	A25	A15
GENERAL		
Name	**ALP-AMP**	**ALP-AMP**
Sample type	SER	SER
Analysis mode	kinetic mon.	kinetic mon.
Units	U/L	U/L
Turbidimetry test	no	No
Decimals	0	0
Type of reaction	increasing	increasing
PROCEDURE		
Reading mode	monoch.	monoch.
Main filter	405	405
Reference filter	-	-
Sample	6	6
Vol. R1	300	300
Vol. R2	-	-
Washing	1.2	1.2
Reading 1 (cycle)	5	4
Reading 2 (cycle)	14	10
Reagent 2 (cycle)	-	-
Predilution factor	-	-
CALIBRATION AND BLANK		
Calibration type	multiple	multiple
Number of calibrators	-	-
Calibration curve	-	-
OPTIONS		
Blank absorbance limit	1.200	1.200
Kinetic blank limit	-	-
Linearity limit	1200	1200
Substrate depletion	-	-

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