



ALKALINE PHOSPHATASE (ALP)

Colorimetric Method (AMP Buffer)

E.C.3.1.3.1.

Cat.No. 101-0020

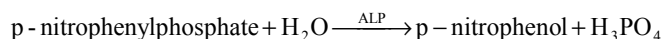
Size 10x10 ml

Cat.No. 101-0086

Size 4x50 ml

PRINCIPLE:

Alkaline phosphatase hydrolyzes p - nitrophenyl phosphate to p - nitrophenol and inorganic phosphate. The rate phosphate (in alkaline solution) of p - nitrophenol, which exhibits an absorbance maximum at 405 nm, is directly proportional to the alkaline phosphatase activity.



SAMPLE:

Serum; plasma (Na-heparin); Stability 7 days at +2 °C to +8 °C or few months at - 20 °C.

REAGENTS:

	Concentrations in the test
1. Substrate p-nitrophenylphosphate	16 mmol/L
2. Buffer, pH 10.5 2-amino-2-methyl-1-propanol	0.9 mol/L
2. Mg ⁺⁺	1.0 mmol/L

Store at +2 °C to +8 °C.

PREPARATION OF REAGENTS:

- Substrate:
Reconstitute the substrate (vial 1) with the corresponding volume of buffer (vial 2):
a) 10 ml for the 10x10 ml kit (Cat.No. 101-0020)
b) 50 ml for the 4x50 ml kit (Cat.No.101-0086)

Solution is stable 1 week at +2 °C to +4 °C or 1 day at room temperature.
- Buffer is ready for use and stable up to the expiry date.
- Solution of magnesium (vial 3) is ready for use and stable up to the expiry date.

Preparation of working reagent:

Add the appropriate volume of solution 3 (Mg⁺⁺) to the contents of one bottle of substrate solution:

- 100 µl for the 10x10 ml kit (Cat.No. 101-0020)
- 500 µl for the 4x50 ml kit (Cat.No. 101-0086)

Working reagent is stable 7 days at +2 °C to +8 °C or 1 day at +15 °C to +25 °C.

PROCEDURE:

Wavelength:	405 nm
Cuvette:	1 cm light path
Temperature:	30 °C, 37 °C
Zero:	H ₂ O

Pipette into cuvette	Macro	Micro
Sample	50 µl	20 µl
Working reagent	2.5 ml	1.0 ml
Mix well, incubate for 30 sec and then read initial absorbance and read absorbance every 60 sec during 3 min. Calculate ΔA/min.		

CALCULATION:

$$\frac{\Delta A/\text{min} \times 10^6 \times TV}{18.6 \times 10^3 \times l \times V} = \Delta A/\text{min} \times 2742 = U/L$$

Where is :

ΔA	= change in absorbance
min	= minute
18.6 x 10 ³	= molar absorptivity of p-nitrophenol at 405 nm
10 ⁶	= conversion of mol in µmol
l	= light path in cm
TV	= total reaction volume in ml
V	= sample volume in ml

$$\text{nkat/L} = U/L \times 16.67$$

EXPECTED VALUES:

	30 °C	37 °C	UNIT
Children	88 – 238	130 – 350	U/L
	1467 - 3967	2167 - 5837	nkat/L
Men	30 – 90	44 – 132	U/L
	500 - 1500	733 - 2200	nkat/L
Women up to 40 years	30 – 90	44 – 132	U/L
	500 - 1500	733 - 2200	nkat/L
Women over 40 years	30 – 100	44 – 147	U/L
	500 - 1667	733 - 2450	nkat/L

LINEARITY:

up to 680 U/L at 30 °C (11335 nkat/L)
up to 1000 U/L at 37 °C (16670 nkat/L)

NOTE:

- Avoid hemolysis as it interferes with the assay.
- Fluoride and anticoagulants such as citrate, EDTA and oxalate should not be used since these agents inhibit alkaline phosphatase activity.
- Solution 2 (buffer) contains sodium azide. Avoid ingestion or contact with skin or mucous membranes.
- During the course of the reaction p-nitrophenol is liberated from the substrate. Avoid contact with skin and do not swallow or inhale vapor.

QUALITY CONTROL:

CONTRO-N	20 x 5 ml	Cat. No. 101-0083
CONTRO-P	20 x 5 ml	Cat. No. 101-0084

REFERENCE:

- Bowers, G.N. and Mc Comb R.B., Clin. Chem. 21,198,1975