

ALKALINE PHOSPHATASE REAGENT KIT

(PNPP Kinetic Method)

Quantitative determination of alkaline phosphatase activity in human serum

Only for In Vitro diagnostic use

Ref no.

ALKP25

ALKP125

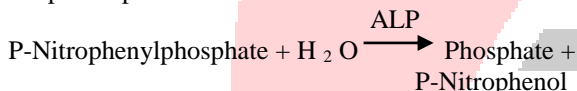
Summary

ALP is an enzyme of the Hydrolase class of enzymes and acts in an alkaline medium. It is found in high concentrations in the liver, biliary tract epithelium and in the bones. Normal levels are age dependent and increase during bone development. Increased levels are mainly associated with liver and bone disease. Moderate increases seen in Hodgkins disease and congestive heart failure.

Principle

ALP at an alkaline pH hydrolyses P-Nitrophenylphosphate to form P-nitrophenyl and Phosphate. The rate of formation of P-nitrophenyl measured as an increase in absorbance which is proportional to the ALP activity in the sample.

The enzymatic reaction sequence employed in the assay of Alkp-Phosphatase is as follows:



Kit Contents

Kit size	25ml	125ml
Ref no.	ALKP25	ALKP125
ALKP-R1	1 x 20ml	2 x 50ml
ALKP-R2	1 x 5ml	1 x 25ml
IFU	1	1

Material required not provided

Test tubes, yellow tips, blue tips.

Storage & Stability of the Reagents

1. The reagents are stable till the date of expiry, when stored at 2^o-8^o C, protect from light & contamination is avoided.
2. Do not freeze the reagents.
3. Always use fresh test tubes & tips.
4. Keep always the tightly caps closed.
5. Ensure the reagents & specimens are brought to Room Temperature.
6. Ensure the reagents shelf life is valid.
7. Do not use haemolysed & lipemic serum.

Reagent preparation

R1= Buffer Reagent

R2= Substrate Reagent

Mix, 4 parts of reagent 1 & 1 part of reagent 2 = working reagent.

The stability of the working reagent is

5 days at 15^o-25^oC

2 Weeks at 2^o-8^oC.

The substrate Reagent (R2) is light and temperature sensitive.

Protect the reagent from light.

Reagent composition

Reagent 1	Diethanolamine	98.6ml/l
	HCL	7.5ml/l
	Mgcl ₂	0.10g/l
Reagent 2	Phenol	10ml/l
	PNPP	2.5g/l

Specimen

Serum

Specimen collection

1. Fresh, clear, non-hemolysed serum from fasting patients is recommended.
2. Oxalate, fluoride & EDTA inhibit Alkp-Phosphatase, hence unsuitable as anticoagulants.
3. Perform the assay as early as possible. Alkp-Phosphatase in serum, or in reconstituted controls, rise significantly when stored.

Storage & Stability of the Specimen

It is best to measure enzyme activity within few hours of sample collection. There is <10% loss of activity within 3 days at at 2^o-8^oC & <17% at 15^o-25^oC.

Warning & Precautions

1. Keep out of reach children. In case of contact with eyes, rinse immediately with plenty of water & seek medical advice.
2. Take off immediately all contaminated clothing.
3. Wear suitable gloves and eye /face protection.
4. Always use safety pipettes to pull the reagents into a pipette.
5. Reagents may contain some non-reactive and preservative components. It is suggested to handle carefully, avoid direct contact with skin and do not swallow.
6. Perform the test according to the current "Good Laboratory Practice"(GLP) guidelines.



SWEMED DIAGNOSTICS

7. The reagents contain sodium azide (0.95g/L) as preservative. Do not swallow. Avoid contact with skin and mucous membrane.

Assay procedure

Wave length	: 405 nm
Temperature	: 37° c
Light path	: 10 mm

Pipette into cuvettes	Macro	Semi-Micro
Reagent (R1+R2)	800µl +200µl	400µl+100µl
Sample	20µl	10µl

Mix well & read the initial absorbance A0 after 1 minute and repeat the absorbance reading after every 1, 2, & 3 minutes. Calculate the mean absorbance change per minute. ($\Delta A/\text{min}$)

Calculation

$$\text{Alkp-Phosphatase (U/L)} = \Delta A/\text{min.} \times 2757$$

Performance Characteristics

Linearity

The procedure is linear upto 700U/L at 37°C, if the absorbance change ($\Delta A/\text{min.}$) exceeds 0.250, use only the value of the first 2 minutes to calculate the result or dilute the sample 1+9 with the normal saline (Nacl 0.9%) and repeat the assay (Result $\times 10$).

Note

Samples having a very high activity show a very high initial absorbance. If this is suspected then dilute the sample and repeat the assay.

Normal Reference values

Serum	Adults	80-290U/L
	Children	245-770U/L

“It is recommended that each laboratory establish its own normal range representing its patient population.”









Quick References

Parameter	Alkaline phosphatase
Mode	Kinetic
Wavelength	405nm
Unit	IU/L
Temperature	37°C
Factor	2757
Reaction slope	Increasing
Reagent volume	1000µl
Sample volume	20µl
Reaction time	180sec
Delay time	60sec
Delta time	60sec
Blanking	Water blank
linearity	700 U/L

Literature

- Z. Klin Chem. U. Klin. Biochem. 8 (1970), 658; (1971) 464; 10 (1972) 182.
- Weishaar, D., Grossau, E., U. Faderl; B. Med. Welt 26 (1975), 387-390.
- Kubler, W. Symp. D. Deutschen Ges. For Lab. Med; Mainz (1973).
- Thomas, L. Labor U/ Diag. 1. Aufl. (1978) S. 64 Die. Med. Verlag, Marburg.
- Schlebusch, H., Rick, W., Lang, H.U. Knedel, M. Dtsch. Med. Wschr. 99 (1974), 765.
- Rick, W., Klinische Chemie and Mikroskopie, P. 294, 6th Edition, springer Verlag, Berlin (1990).

Note on symbols and marks

 Instructions for use	 Use by	 Batch number	 Manufacturer
 Invitro Diagnostic Medical Device	 Date of manufacturer	 Temperature limit	 Reference number