ALKALINE PHOSPHATASE KIT LS

(pNPP Kinetic Method)

For the determination of Alkaline Phosphatase activity in serum.

(For Invitro Diagnostic Use Only)

Summary

Alkaline Phosphatase (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 associated mainly with liver and bone disease. Moderate increases are seen in Hodgkins disease and congestive heart failure.

Principle

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

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

Normal reference values

Serum (Adults) : 80 - 290 U/L at 37°C
(Children) : 245 - 770 U/L at 37°C

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

Contents	25 ml	75 ml	2 x 150 ml
L1 : Buffer Reagent	20 ml	60 ml	2 x 120 ml
L2 : Starter Reagent	5 ml	15 ml	2 x 30 ml

Storage / stability

Contents are stable at 2-8° C till the expiry mentioned on the labels.

Reagent Preparation

Reagents are ready to use.

Working reagent : For sample start assays a single reagent is required. Pour the contents of 1 bottle of L2 (Starter Reagent) into 1 bottle of L1 (Buffer Reagent). This working reagent is stable for at least 2 week when stored at 2-8° C.

Alternatively for flexibility as much of working reagent may be made as and when desired by mixing together 4 parts of L1 (Buffer Reagent) and 1 part of L2 (Starter Reagent). Alternatively 0.8 ml of L1 and 0.2 ml of L2 may also be used instead of 1 ml of the working reagent directly during the assay.

Sample material

Serum. Free from hemolysis. ALP is reported to be stable in serum for 3 days at 2-8° C.

Procedure

Wavelength / filter : 405 nm
Temperature : 37° C / 30° C / 25° C
Light path : 1 cm

Substrate Start Assay :

Pipette into a clean dry test tube labelled as Test (T) :

Addition Sequence	(T) 37° C
Buffer Reagent	0.8 ml
Sample	0.02 ml
Incubate at the assay temperature for 1 minute and add	
Starter Reagent	0.2 ml

Mix well and read the initial absorbance A_0 after 30 seconds & repeat the absorbance reading after every 1 & 2 minutes. Calculate the mean absorbance change per minute (ΔA / min.)

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Calculations

ALP Activity in U/L = ΔA / min. x 2754

TEMPERATURE CONVERSION FACTORS

Assay Temperature	Desired Reporting Temperature 25° C	30° C	37° C
25° C	1.00	1.22	1.64
30° C	0.82	1.00	1.33
37° C	0.61	0.75	1.00

Linearity

The procedure is linear upto 700 U/L at 37° C. If the absorbance change (ΔA / min.) exceeds 0.250, dilute the sample 1 + 9 with normal saline (NaCl 0.9%) and repeat the assay (Results x 10).

Notes

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

The substrate is light and temperature sensitive. Take adequate care, especially after reconstitution.

References

Bowers, G.N., McCommb, R.B., (1972) Clin. Chem. 18 : 97

Recommendations of the German Society for Clinical Chemistry, (1972)
Z. Clin. Chem. Bio. 10 : 182

System Parameters

Reaction	:	Kinetic
Wavelength	:	405 nm
Zero Setting	:	Distilled water
Incub. Temp.	:	37^{0}C
Incub. Time	:	---
Delay Time	:	30 sec.
Read Time	:	120 sec.
No. of read.	:	4
Interval	:	30 sec.
Sample Vol.	:	0.02 ml
Reagent Vol.	:	1.00 ml
Standard	:	---
Factor	:	2754
React. Slope	:	Increasing
Linearity	:	700 U/L
Units	:	U/L

ALP(LS):00(P)

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