ALKALINE PHOSPHATASE KIT (DEA)

(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	10 x 3 ml	5 x 15 ml	20 x 15 ml
L1 : Buffer Reagent	35 ml	80 ml	2 x 150 ml
T1 : Substrate Reagent	10 Nos.	5 Nos.	2 x 10 Nos.

Storage / stability

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

Reagent Preparation

Working reagent : Dissolve 1 substrate tablet in 3.2 ml (10 x 3 ml pack) or 15 ml (5 x 15 ml / 20 x 15 ml pack) of buffer reagent.

This working reagent is stable for at least 15 days when stored at 2-8°C.

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

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

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

Addition Sequence	(T) (ml)
Working Reagent	1.0
Incubate at the assay temperature for 1 minute and add	
Sample	0.02

Mix well and read the initial absorbance A_0 after 1 minute & repeat the absorbance reading after every 1, 2, & 3 minutes. Calculate the mean absorbance change per minute (Δ A / min.)

Calculations

ALP Activity in U/L = $\Delta A / \text{min.} \times 2754$

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, use only the value of the first two minutes to calculate the result, or dilute the sample 1 + 9 with normal saline (NaCl 0.9%) and repeat the assay (Results x 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.

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(DEA)\02(P)

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