Asritha
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ALKALINE PHOSPHATASE KIT

(PNPP Kinetic Method)
For the determination of Alkaline Phosphatase activity in serum.
(For In vitro Diagnostic Use Only)

CLINICAL SIGNIFICANCE

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.

INCREASES

Increased levels are associated mainly with liver and bone disease. Moderate increases are seen in Hodgkins disease and congestive heart failure.

METHODOLOGY : PNPP Kinetic Method

PRINCIPLE

$$\text{p-Nitrophenylphosphate} + H_2O \xrightarrow{\text{ALP}} \text{p-Nitrophenol} + \text{Phosphate}$$

REAGENT COMPOSITION

pNPP	:	12 mmol/L
Mg^{+2}	:	10 mmol/L
DEA Buffer	:	80 mmol/L

STORAGE AND STABILITY

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

REAGENT PREPARATION

Reagents are ready to use.

WORKING REAGENT

Dissolve 1 substrate vial in 5ml (7x5ml pack) or 10 ml (6x10ml pack) of buffer reagent. This working reagent is stable for atleast 45 days when stored at 2-8°C.

The Substrate is light & 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.

ASSAY PARAMETERS

Reaction	Kinetic	Interval	60 sec.
Wavelength	405 nm	Sample Vol.	0.02 ml
Zero Settings	Distilled Water	Reagent Vol.	1.00 ml
Incub. Temp	37°C	Standard	-
Incub Time	-	Factor	2754
Delay Time	60 sec.	React. Slope	Increasing
Read Time	-	Linearity	1000 IU/L
No. of read.	4	Units	IU/L

ASSAY 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 ml
Incubate at the assay temperature for 1 min. and add	
Sample	0.02ml

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 IU/L = ΔA / min x 2754 x tf

TEMPERATURE CONVERSION FACTORS

Assay Temperature	FACTOR TF
25° C	1.7
30° C	1.34
37° C	1.00

LINEARITY

The procedure is linear upto 1000 IU/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.

Adherence to the reaction time should be meticulously followed.

Substrate Reagent and Working Reagent should not be exposed to light or left at R.T.

QUALITY CONTROL

To ensure adequate Quality control each run should include assayed Normal and abnormal controls.

NORMAL REFERENCE VALUES

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

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

REFERENCES

1. Bowers, G.N.McCommb, R.B.(1972) Clin. Chem. 18:97
2. Recommendations of German society for clinical Chemistry, (1972)
3. Z. Clin. Chem. Bio. 10:182

PRESENTATION

PRODUCT CODE	PACK SIZE	BUFFER REAGENT (A_1)	SUBSTRATE REAGENT (A_2)
AAL 0601	7 x 5 ml	1 x 35 ml	7 Nos. x 5 ml
AAL 0602	6 x 10 ml	1 x 60 ml	6 Nos. x 10 ml

PRODUCT FEATURES AT A GLANCE :

1. **Simple and one step Kinetic assay.**
2. **Can be used in all Semi and fully automated analyzers.**
3. **Reconstituted stability 45 days at 2 – 8°C.**
4. **Extremely stable reagent blanks.**
5. **Highest Linearity 1000 IU/L.**
6. **Convenient pack size – 7 x 5 ml, 6 x 10 ml.**
7. **Store at 2-8°C.**

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ASRITHA DIATECH INDIA PVT. LTD.

IN VITRO DIAGNOSTIC REAGENTS

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