

LIQVIPATH ALKALINE PHOSPHATASE

(pNPP-DEA METHOD)

CAT NO.: ALL

Reagent kit for quantitative estimation of Alkaline Phosphatase activity in Serum or Plasma.

DIAGNOSTIC SIGNIFICANCE:

Alkaline Phosphatase is present in high concentrations in the liver, bone, placenta, intestine and certain tumors. Increase in Alkaline Phosphatase activity in serum or plasma is related to diseases of bone, biliary tract and liver. Decrease in activity is found in severe anemia, scurvy, kwashiorkar & cretinism.

PRINCIPLE:

Alkaline Phosphatase (ALP) in a sample hydrolyses paranitrophenyl phosphate (pNPP) into paranitrophenol & phosphate, in the presence of magnesium ions. The rate of increase in absorbance of the reaction mixture at 405 nm due to liberation of paranitrophenol is proportional to the alkaline phosphatase activity.

pNPP + H₂O ALP Paranitrophenol + Phosphate

SPECIMEN COLLECTION:

Fresh Serum free from hemolysis. Plasma collected using heparin as an anticoagulant may be used. Avoid anticoagulants like oxalate, citrate, and EDTA.

KIT PRESENTATION:

Pack Size	2 X 10 ml	2 X 25 ml	2 X 50 ml
R1- Alk.PO ₄ Tase (Buffer)	2 X 08 ml	2 X 20 ml	2 X 40 ml
R2- Alk.PO ₄ Tase (Substrate)	2 X 02 ml	2 X 05 ml	2 X 10 ml

WORKING REAGENT PREPARATION:

Mixing 4 volumes of R1 (Buffer) with 1 volume of R2 (Substrate). i.e. 800 µl R1 + 200 µl R2.

The working reagent is stable for **30 days** at 2-8°C.

REAGENT STORAGE AND STABILITY:

All reagents are stable at 2-8°C until the expiry date stated on the label.

ASSAY PARAMETERS:

Reaction	: Kinetic	Sample Volume	: 20 µl
Wavelength	: 405 nm	R1 + R2 Volume	: 800 µl + 200 µl
Flow Cell Temp.	: 37ºC	Factor	: 2750
Initial Delay	: 30 Sec	Reaction Slop	: Increasing
Interval Time	: 30 Sec	Zero Setting	: Dist. Water
Read Time	: 90 Sec	Linearity	: 2000 IU/L
No. of Reading	: 03	Unit	: IU/L

PROCEDURE:

Addition Sequence	Test
R1- Alkaline Phosphatase (Buffer Reagent)	800 µl
R2- Alkaline Phosphatase (Substrate Reagent)	200 µl
Sample (Test)	20 µl

Mix & aspirate immediately and read first absorbance of test exactly at 30 seconds and then, second, third and fourth at an interval of 30 seconds at 405 nm. Determine the mean change in absorbance per minute. ($\Delta A/min$) and calculate the test results.

CALCULATION:

Alkaline Phosphatase Activity (IU/L) = $\Delta A/\min X$ 2750

NORMAL VALUES:

• CHILDREN (3-15 YEARS) : 245 - 770 IU/L Female : 64 - 306 IU/L Male : 80 - 306 IU/L

Each laboratory should establish its own reference range.

LINEARITY:

This method is linear up to 2000 IU/L. For values above 2000 IU/L, dilute the sample suitably with 0.9 % saline, and repeat the assay. Apply correction due to dilution to arrive at a final result.

REFERENCES:

Recommendations of the German Society for Clinical Chemistry: Standardization of Methods for the estimation of Enzyme Activity in Biological Fluids, J. Clinical Chemistry, Clinical Biochemistry 8. 182 -192 (1972).

IFU No.: 003/00 Rev. No.: 00/120723

IVD In-Vitro Diagnostics Use

Mfg. Date

LOT Batch Numbe

REF Catalogue Number

 \mathbf{i} See Package Insert