

# ALKALINE PHOSPHATASE

METHOD – PNPP – DIETHANOLAMINE (DEA) METHOD  
PRODUCT CODE – DA01



## INSTRUCTIONS FOR USE

**INTENDED USE:** Test for estimation of Alkaline Phosphatase activity in serum / plasma using PNPP kinetic method.

### SUMMARY AND PRINCIPLE

1. Serum Alkaline Phosphatase levels are of interest in the diagnosis of hepatobiliary disorders and bone disease associated with increased osteoblastic activity. Moderate elevations of Alkaline Phosphatase may be seen in Hodgkin's disease, congestive heart failure, ulcerative colitis, regional enteritis, and intra-abdominal bacterial infections.
2. *Alk.Phosphatase* is a reagent set for determination of Alkaline Phosphatase activity based on kinetic method using P-nitrophenylphosphate.
3. *Alk.Phosphatase* is a single reagent system with one step reconstitution.



### PREPARATION, STORAGE & STABILITY

Reconstitute each substrate tablet with diluent as per the instruction indicated on the substrate bottle. The working reagent is stable for 21 days at 2- 8 °C. The reagent kit should be stored at 2- 8 °C and is stable till the expiry date indicated on the label.

### TEST PARAMETERS

Name	ALK.PHOS.	Reagent Vol	1000 µl
Reaction Type	Kinetic (↑)	Sample Vol	20 µl
Wavelength	405 nm	Temperature	37 °C
Flow Cell Temp.	37 °C	Delay Time	60 sec.
Blank setting	Reagent	Read Time	30 sec.
Blank abs. limit	< 0.700	Factor	2720
Linearity	2000 IU/L	Standard Conc.	-

### ASSAY PROCEDURE

	Test
Reagent	1000 µl
Serum / Plasma	20 µl
Mix the reagent and sample in the above mentioned ratio and start the stop watch.	
Aspirate reaction mixture into flow cell.	
Record absorbance at 60 <sup>th</sup> and 90 <sup>th</sup> sec.	

### CALCULATION

Calculate average  $\Delta \text{Abs}/\text{min} = \Delta \text{Abs} / 30 \text{ sec} \times 2$   
Alkaline Phosphatase (IU/L) =  $\Delta \text{Abs} / \text{min} \times 2720$

### COMPONENTS OF REAGENT

Component	Concentration
Diethanolamine Buffer , pH 9.8	1 Mol/l
p-nitrophenyl phosphate	10 mMol/l
Magnesium chloride	0.5 mMol/l
Stabilizers and inactive ingredients.	-

### SPECIMEN COLLECTION & PRESERVATION

Blood should be collected in a clean dry container. Hemolysed specimen should be avoided as it may falsely elevate results. EDTA, Citrate and Oxalate inhibit Alkaline Phosphatase activity and should not be used as anti-coagulant. Alkaline Phosphatase in the serum/plasma is stable for 4 days when stored at 2- 8 °C and several months when stored at -10° C.

### REFERENCE VALUES FOR NORMAL PEOPLE

Adults (>15 Years) – 108 – 306 IU/L  
Children (<15 Years) – 210 – 810 IU/L

### QUALITY CONTROL

1. Inclusion of a normal value and abnormal value chemistry control serum in each test run ensures optimum quality control.
2. Consistent use of same type and methodology of control serum provides between run precision and accuracy data for Alkaline Phosphatase. We recommend to produce such data on daily basis for greater accuracy in assay system which include reagents, instrument, apparatus and operator.

### PRECAUTIONS

1. Discard the reagent if its absorbance exceeds 0.700 at 405 nm against distilled water.
2. If Alkaline Phosphatase activity exceeds 2000 IU/L then dilute the specimen suitably with normal saline & repeat the assay. In such case the results obtained should be multiplied by dilution factor to obtain the correct Alkaline Phosphatase activity.

### BIBLIOGRAPHY

1. Henry R.J., "Enzymes" in Clinical Chemistry Principle and Techniques,
2. Young D.S. et al, Clin Chem. 18, 1041, (1972)

### SYMBOLS:



Read Instruction for use



In Vitro Diagnostic Use Only



Manufactured by



Expiry Date



Storage Temperature

ANAMOL LABORATORIES PVT. LTD.  
61, Genesis Industrial Township, Kolgaon,  
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ISO 13485 : 2003  
GMP  
CE