

**(p- NPP METHOD with stabilised substrate)**

**Reagent kit for quantitative estimation of Alkaline Phosphatase activity in serum or plasma.**

- Long shelf life
- High stability (Tableted Substrate)
- Optimised formulation as per GSCC
- Convenient Pack sizes
- Accuracy & reproducibility (precision) of International Standard

**INTRODUCTION :**

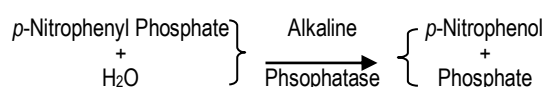
**ENZOPAK** Alkaline Phosphatase is a kinetic procedure based on the recommendation by German Society for Clinical Chemistry (GSCC).

Most of the formulations by commercial manufacturers use substrate as p-NPP disodium hexahydrate salt which deteriorates faster than p-NPP used in our formulation. The deterioration increases free p-NP, resulting in higher blanks. Our stabilised p-NPP resists deterioration and keeps the blank low all throughout the shelf life.

The concentrations of the diethanolamine (DEA) buffer and substrate (p-NPP) in the reagent system estimate the phosphate transferase activity optimally, the DEA buffer system being a phosphate acceptor.

**PRINCIPLE :**

Alkaline Phosphatase in a sample, hydrolyses para-nitrophenyl phosphate into paranitrophenol and 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.



**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, kwashiorkor & cretinism.

**REAGENT COMPOSITION :**

Active Ingredients	Concentration
<b>Reagent-1</b>	
• p-NPP	16.3 mmol/L
• Sodium chloride	1000 mmol/L
<b>Reagent-2</b>	
• Buffer	1000 mmol/L
pH 9.9± 0.1 at 25°C	
Also contains non-reactive fillers and Stabilizers.	

**PRESENTATION :**

	No. of Bottle/Blister
All reagents to be stored at 2-8°C.	50 x 1.1 ml
• 1 Alkaline Phosphatase (Substrate)	5 (10 Tablets)
• 2 Alkaline Phosphatase (Buffer)	1
• Reconstitution vial	2

**PRECAUTION :**

Alk. Phosphatase is for *IN-VITRO* diagnostic use only.  
**Reagent contains Sodium Azide, DO NOT INGEST.**

**WORKING REAGENT PREPARATION:**

**For 50 x 1.1 ml**

Dissolve one tablet (1 ALK. PHOSPHATASE) in 1.1 ml of buffer (2 ALK. PHOSPHATASE) to make buffered substrate. For quick dissolution crush the tablet prior to addition of buffer. Keep for 15 minutes before use.

**REAGENT STORAGE AND STABILITY :**

ENZOPAK Alkaline Phosphatase reagents are stable till the expiry stated on the label, when stored at 2-8°C.

The buffered substrate should be used on the same day. It may be stored in a refrigerator (2-8°C) for 10 days in a dark coloured bottle. Para-nitrophenyl phosphate in solution slowly breaks down and para-nitrophenol is liberated. If the blank absorbance of the buffered substrate exceeds 0.85 at 405 nm against distilled water, use options given under LINEARITY or discard the working reagent.

**SPECIMEN COLLECTION :**

Fresh, clear serum, under fasting condition with no hemolysis is the specimen of choice.

Plasma collected using heparin as an anticoagulant may be used.

Avoid anticoagulants like oxalate, citrate and EDTA.

**REACTION PARAMETERS :**

- Type of Reaction : Kinetic/Increasing OD
- Wavelength : 405 nm
- Flowcell Temp. : 37°C
- Delay Time : 30 Seconds
- Interval : 30 Seconds
- No. of reading : 4
- Sample volume : 20 microliters (0.02 ml)
- Working Reagent Volume : 1.0 ml
- Factor : 2713
- Light Path : 1.0 cm.
- Zero setting with : Reagent Blank

**PROCEDURE :**

PIPETTE INTO TEST TUBES	TEST
• BUFFERED SUBSTRATE (ml)	1.0
• SAMPLE (ml)	0.02

Mix and read absorbance at 30, 60, 90 and 120 seconds at 405 nm. Determine the mean change in absorbance per minute and calculate test results.

**TEST RESULTS :**

Alkaline Phosphatase activity (IU/L) = ΔA/min x F  
 Where F = 2713 (calculated on the basis of molar extinction coefficient for p-nitrophenol and ratio of total assay volume to sample volume).

**NORMAL VALUES :**

	At 37°C
CHILDREN (3-15 YEARS)	: 250-770 IU/L
ADULTS	: 100-250 IU/L



## LINEARITY :

Method is linear upto 1500 IU/L. For sample value exceeding the linearity limit, dilute samples suitably with 0.9 % saline and repeat the assay. Apply dilution factor to calculate the test results.

For reconstituted reagent stored for more than 3 days at 2-8°C the following guidelines may be used for serum samples of very high enzyme activity:

- (a) If the initial absorbance is between 0.85 to 1.0 then the number of readings may be reduced to 2 with an interval of 30 seconds i.e. upto 90 seconds only.
- (b) If the initial absorbance is between 1.0 to 1.2 then the number of readings may be reduced to 1 with an interval of 30 seconds i.e. upto 60 seconds only.

## NOTE :

For laboratories using instruments with cuvette capacity more than 1.0 ml, sample and buffered substrate volumes should be increased proportionally.

## 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)

