

# ALKALINE PHOSPHATASE (DST)

**ENZOPAK**

Last update 04-2014

## (p-NPP METHOD with stabilised substrate)

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

- Long Shelf Life
- High stability
- Scientific optimized formulation as per GSCC
- Pack sizes suitable for every laboratory
- Accuracy & reproducibility (precision) As per International Standard.
- Very well accepted by good customers

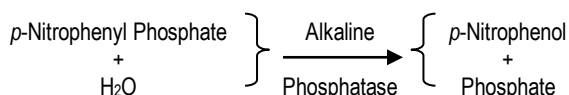
## INTRODUCTION:

ENZOPAK Alkaline Phosphatase is a kinetic procedure based on the recommendations by German Society for Clinical Chemistry (GSCC). Most of the formulations by commercial manufacturers use substrate as p-NPP disodium hexahydrate, 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 concentration 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 para-nitrophenol 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 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	20 mmol/L
• Sodium chloride	500 mmol/L
<b>Reagent-2</b>	
• Buffer	800mmol/L
pH 9.9± 0.5 at 25°C	
Also contains non-reactive fillers and Stabilizers.	

## PRESENTATION :

	Pack Size	
Store all reagents at 2-8°C	10 x 10 ml	10 x 5 ml
1. Alk. Phosphatase (Substrate)	1 (10 Tablet)	1 (10 Tablet)
2. Alk. Phosphatase (Buffer)	2	1
Reconstitution bottle	1	1

## PRECAUTION :

ENZOPAK Alkaline Phosphatase is for *IN-VITRO* diagnostic use only.

**Reagent contains Sodium Azide, DO NOT INGEST.**

## WORKING REAGENT PREPARATION

### For 10 x 10 ml :

Carefully transfer one tablet of 1 alkaline phosphatase into the bottle containing 10 ml of 2 Alkaline Phosphatase (buffer). Mix gently to dissolve completely Wait for 15 minutes before use.

### For 10 x 5 ml:

Carefully transfer one tablet of 1 alkaline phosphatase into the bottle containing 5 ml of 2 Alkaline Phosphatase (buffer). Mix gently to dissolve completely Wait 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 at 2-8°C for 30 days in a dark coloured bottle (provided in the kit). Para-nitrophenyl phosphate in solution slowly breaks down and paranitrophenol 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.

## GENERAL PARAMETERS :

• Type of Reaction	:	Kinetic/Increasing OD
• Wavelength	:	405 nm
• Flowcell Temperature	:	37°C
• Delay Time	:	30 Seconds
• Interval	:	30 Seconds
• No. of readings	:	4
• Sample Volume	:	20 µl (0.02 ml)
• Working Reagent Volume	:	1.0 ml
• Factor	:	2713
• Light Path	:	1.0 cm
• Zero setting with	:	Distilled Water

## 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) =  $\Delta A/\text{min.} \times F$   
 Where F = 2713 (calculated on the basis of molar extinction coefficient for p-nitrophenol and ratio of total assay volume to sample volume).



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## NORMAL VALUES:

- CHILDREN : 250-770 IU/L
- (3-15) YEARS : } At 37°C
- 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 result.

For reconstituted reagent stored 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 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 seconds only.

## NOTE :

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

## 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. 192-192 (1972).

