



ALKALINE PHOSPHATASE

(PNPP Method)

Liquid Reagent

INTENDED USE:

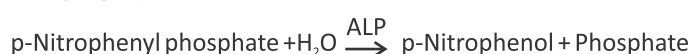
This reagent kit is used for *in-vitro* quantitative determination of Alkaline Phosphatase (ALP) in human serum/plasma.

TEST PRINCIPLE:

Under alkaline condition, colorless p-Nitrophenyl phosphate is converted to phosphate and p-nitrophenol which develops a very intense yellow color.

Its intensity is proportional to the activity of alkaline phosphatase in the sample.

REACTION :



KIT CONTENTS:

- Reagent 1** : AMP Buffer
Reagent 2 : PNPP Substrate
Product Insert : 01 No.

PREPARATION OF THE WORKING REAGENT:

Mix 4 parts of reagent 1 with 1 part of reagent 2

REAGENT STABILITY AND STORAGE:



Conditions : protect from light and close immediately after use.

Working Reagent:

Stability : 30 days at 2-8°C & 5 days at 21-25°C

Maximum allowable absorbance of working reagent measured at 405 nm against water as reference is 0.8.

SAMPLE COLLECTION AND STORAGE:

Unhaemolysed serum, ALP is reported to be stable in serum for 3 days at 2-8°C and 1 day at 21-25°C.

PRECAUTIONS: ⚠

- Storage conditions as mentioned on the kit to be adhered.
- Do not freeze or expose the reagents to higher temperature as it may affect the performance of the kit.
- Before the assay bring all the reagents to room temperature.
- After use store the kit contents immediately as 2-8°C.
- Avoid contamination of the reagent during assay process.
- Use clean glassware free from dust or debris.
- Reagent ratio as mentioned here above must be strictly observed as may change into it will adversely effect the factor.
- Do not use the reagent if the reagent is hazy or cloudy.

PROCEDURE (Automated):

Refer to specific instrument application instructions.

TEST PROCEDURE (Manual) :

Wavelength : 405 nm & Reaction Temperature : 37°C

Pipette into Test Tube	Test
Working Reagent	1 ml
Sample	20 µl
Mix and after 1 minute incubation, measure change in absorbance (ΔOD/min) for 3 minute and use it for calculation.	

CALCULATIONS:

Alkaline Phosphatase activity (IU/l) = ΔOD/min X 2764 (Factor)

NORMAL VALUES*:

- Children : 104 - 390 IU/l
Adult : 25 - 140 IU/l

*It is recommended that each laboratory should establish its own normal range.

PERFORMANCE:

- Linearity:** 1600 IU/l
- Comparison:** $r = 0.98$
 $y = 0.98x - 1.9$

3. Precision:

	Within Run			Run to Run		
	Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
Low	45.0	0.4	0.9	51.0	1.9	1.8
High	145.0	0.9	0.4	149.0	1.4	0.9

4. Specificity:

Anticoagulants other than heparin, ammonium and phosphate salts, improperly cleaned glassware may inhibit alkaline phosphatase activity.

CLINICAL SIGNIFICANCE:

Elevated level of serum alkaline phosphatase is associated with bone degeneration and the activity increases in rickets and osteomalacia. Higher values of activity are also found in post hepatic jaundice and infective or toxic hepatitis. Elevated levels of activity also occur during pregnancy and due to intake of certain drugs like steroids, sulphonamides etc.

AUTOMATED APPLICATIONS:

Alkaline Phosphatase Liquid reagents can be used with most of the commonly available semi-auto and fully-automated biochemistry analyzers. Application sheets for use on specific semi-automatic, batch analyzers are available on request.

Input parameters for semi- auto/auto analyzers are given below.

INPUT PARAMETERS	VALUES
Type of reaction	Kinetic
Slope of reaction	Increasing
Wavelength	405 nm
Incubation time	60 sec.
Interval time	60 sec.
No. of Reading	3
Factor	2764
Temperature	37°C
Units	IU/l
Upper Normal value	390 IU/l
Lower Normal value	25 IU/l
Linearity	1600 IU/l
Reagent volume	1.0 ml
Sample volume	20 µl

QUALITY CONTROL:

For accuracy, it is necessary to run known serum controls with each assay.

REFERENCES:

1. Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-BooksVerlagsgesellschaft: 1998. p. 136-46.
2. Moss DW, Henderson R. Clinical enzymology. In: Burtis CA, Ashwood ER. eds. Tietz textbook of clinical chemistry. 3rd ed. Philadelphia: W. B. Saunders Company, 1999. p. 617-721.
3. Fischbach F, Zawta B. Age-dependent reference limits of several enzymes in plasma at different measuring temperatures. Klin Lab 1992;38:555-61.