ALKALINE PHOSPHATASE – LR

(p-NPP - DEA Kinetic Method)

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INTENDED FOR USE:

The Alkaline phosphatase - LR is an *in vitro* assay for the quantitative determination of alkaline phosphatase in serum, plasma.

CLINICAL SIGNIFICANCE:

Alkaline Phosphatase (ALP) belongs to the Hydrolase class of enzymes and catalyzes the splitting of organic phosphate esters, with optimum activity at pH 10.0. The main site of synthesis of this enzyme is hepatocytes adjacent to biliary canaliculi and active osteoblast. However, liver, bone and placenta contain very high concentrations of ALP. Hence serum ALP measurement is of particular interest in the Hepatobiliary disease and in bone diseases.

Elevated ALP levels are seen in toxic hepatitis, infective hepatitis, intra and extra hepatic obstructions. High ALP levels are also seen in osteomalacia, rickets and bone cancer. The use of p-Nitro phenyl Phosphate (p-NPP) as a substrate for ALP assay produces a chromogenic product, p-Nitro phenol (PNP) which is quantitated directly.

PRINCIPLE:

Alkaline phosphatase reacts with p-nitrophenyl phosphate to form p-nitrophenol, the rate of formation of which is directly proportional to the levels of alkaline phosphates.

$$\text{P-Nitrophenyl phosphate} + H_2O \xrightarrow[\text{DEA}]{\text{ALP, } Mg^{2+}} \text{p-Nitro phenol} + \text{phosphate}$$

REAGENT COMPOSITION:

Alkaline Phosphatase Reagent

DEA Buffer	100 mmol /L
Mg^{2+}	10 mmol /L
P-nitro phenyl phosphate	12 mmol/L
Surfactant, Preservative	qs

REAGENT STORAGE:

All reagents are stable at 2-8°C until the expiry date mentioned on the label. Reagents are ready to use. Before the assay bring all the reagents to room temperature. Avoid contamination of the reagents during the assay process and should be protected from direct light.

SAMPLE COLLECTION:

Unhemolized serum or plasma (heparin)
ALP activity is inhibited by EDTA anticoagulants, oxalates, and citrates. ALP in serum is reported stable up to 3 days at 2-8 °C.

PRECAUTIONS & WARNING:

Avoid pipette with mouth. The preparation, according to current regulation, is classified as not dangerous. The total concentration of non active components (preservatives, detergents, stabilizers) is below the minimum required for citation. Anyway handle with care, avoid ingestion, avoid contact with eyes, skin and mucous membranes. The samples must be handle as potentially infected from HIV or Hepatitis.

SYSTEM PARAMETERS:

Reaction Type	:	Kinetic Method
Wave Length	:	405 nm
Optical path length	:	1 cm
Abs max	:	≤ 1.2
Flow cell Temperature	:	37^0C
Blank	:	Distilled Water
Delay Time	:	30 Sec
Read Time	:	60 Sec
Number of readings	:	4
Working Reagent	:	1000 µL
Sample Volume	:	25 µL
Factor	:	2757
Reaction Slope	:	Increasing
Low Normal	:	0
High Normal	:	280
Linearity	:	1000
Units	:	IU/L

Note:

1. Do not leave the unused reagent at room temperature when not in use. Take only the required amount of the reagent and keep the reagent back immediately at 2-8°C.
2. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
3. Programmers for specific auto analyzers are available on request.
4. p-NPP substrate reagent is highly photo sensitive and should not be exposed to direct sun light.

ASSAY PROCEDURE:

Pipette in to test tubes labeled Test (T) as follows**:**

Addition Sequence	Test (µL)
Alkaline Phosphatase Reagent	1000
Sample	25

Assay temperature 37°C mix well and read absorbance against distilled water at 405nm as Follows:
A_0 - exactly after 30 seconds
A_1 A_2 A_3 exactly after every 1 minute for 3 minutes.
Determine the average change in absorbance per minute (ΔA/min).

Note: Sample having a very high activity show a very initial absorbance. If this is suspected then dilute the sample and repeat the assay. Adherence to the reaction time should be meticulously followed.

CALCULATION:

ALP activity in IU/L = ΔA/min X 2757

SENSITIVITY / LIMIT OF DETECTION:

The method will accurately measure alkaline phosphatase level is 20 IU/L.

LINEARITY:

The procedure is linearity up to 1000 IU/L. If values exceed this limit dilute the sample with normal saline and repeat the assay. Calculate the value using the proper dilution factor.

QUALITY CONTROL:

To ensure adequate quality control, the use of commercial reference control serum is recommended with each assay batch. Use of quality control material checks both the instrument and reagent function

REFERENCE RANGE:

Serum/Plasma
Adults : Up to 280 IU/L
Children : Up to 15 yrs : < 644 IU/L
15 – 17 yrs : < 483 IU/L
Note: It is recommended that each laboratory establish its own normal range representing its patient population.

INTERFERENCE:

No interference was observed by up to ascorbic acid 30 mg/dL, bilirubin 40 mg/dL, hemoglobin 400 mg/dL and triglycerides 500 mg/dL.

PRECISION:

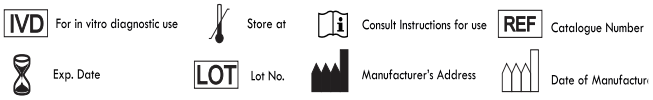
Precision studies were performed with two controls using NCCLS protocol EP5 –A. The results of the precision studies are shown below:

Sample	Within - Run		Between - Run		Total	
	Mean	CV%	Mean	CV%	Mean	CV%
Control-1	171	2.52	163	2.40	334	4.92
Control-2	410	2.14	423	2.04	834	4.17

REFERENCES:

1. Young, D.S. Effects of Drugs on Clinical Laboratory Tests.4th Edition. AACC Press (1995).
2. Tietz. N.W. Clinical Guide to Laboratory Tests, 3rd Edition. W.B. Saunders Co. Philadelphia, PA. (1995).
3. Bowers, G.N. & Mc Comb, R.B. (1972) Clin.Chem.18,97.

SYMBOLS USED ON THE LABELS:



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