ALKALINE
PHOSPHATASE –LS
(P-NPP-DEA METHOD)

PRINCIPLE

Alkaline phosphate catalyses the hydrolysis p NPP to form yellow coloured pNPP. The rate of PNP formation directly propotional to ALP activity.

P-Nitro phenyl phosphate+H2**O** —> Phosphate+p-Nitro phenol

CLINICAL SIGNIFICANCE

Alkaline Phosphatase is an enzyme of the hydrolase class of enzyme and acts in an alkaline medium, The main site of synthesis of this enzyme is hepatocytes adjacent to biliary canalculai and active osteoblast.it is found in high concentrations in the liver, biliary, tract, and epithelium and in the bones.
Normal levels are age dependent and increase during bone development. Increased levels are associated mainly with liver and bone diseases. Moderate increases are seen in Hodgkin's disease andcongestive heart failure
Elevated ALP levels are seen in toxic hepatits, infective hepatits, intra and extra hepatic abstractions.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 quantitated directly.

SPECIMEN COLLECTION

1) Fresh, Fasting Un-hemolysed serum sample is preferred
2) Anticoagulants like oxalates, citrate, AND edta should be avoided.
3) Samples should be assayed on the same day. If necessary they may be preserved up to 72hours if frozen immediately.
4) Avoid use of grossly hemolysed samples.

PRECAUTIONS/NOTE

1) ALKALINE PHOSPHATASE Reagent is for in Vitro diagnostic use only.
2) Bring reagent to room temperature
3) p-NPP substrate reagent is highly photo sensitive and should not be exposed to direct sunlight.
4) 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 at 2-80C immediately.
5) The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
6) if the change absorbance is greater than 0.36/min repeat the assay with iluted samples (iluted with saline) and remember to multiply the final result by the dilution factor.
7) The p-NPP substrate reagent should not be used if the absorbance is more than 1.2.

KIT CONTENTS & STORAGE

Reagent1 . Pnpp substrate reagent 5*10ml , 2-80c

WORKING REAGENT PREPARATION

The reagent is ready to use and is stable at 2-80c till the expiry date mentioned on the label.

SYSTEM PARAMETERS

Reaction type : Kinetic
Wave length : 405nm
Flow cell temp : 370c
Blank : Distilled water
Reagent volume : 1000µl
Sample volume : 25 µl
Delay time : 30sec
Kinetic interval : 60
Factor : 2757
No. of Readings : 4
Reaction incubation : increasing
Abs. Maxim : ≤ 1.2
Units : IU/L
High normal : 280(Adults)

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PROCEDURE

Working reagent	1.0ml
Serum	25 µl

Mix thoroughly and read absorbance against distilled water at 405 nm as follows

A0 - Exactly after 30seconds

A1 A2A3 - Exactly after every one minute for 3 minutes

CALCULATIONS

Conc of Alkaline Phosphatase in Serum (IU/L) = **ΔA/Min x Factor2757**

LINEARITY

This method is linear for ALP up to 1000 IU/L For sample values exceeding the linearity limit, dilute the sample suitably with normal saline and repeat the assay. Apply proper dilution factor while calculation.

NORMAL RANGE

Children up to 15 years old < 644 IU/L
Children 15-17 years < 483 IU/L
Adults up to 280 IU/LDue to variation in inter – laboratory assay conditions, instruments and demography, it is recommended that each laboratory should establish its own normal range.

Bibilography

1. Z.kin.chem.U.Kin.Biochem 8,658(1970)
2. RICK,W,Kinische Chemie and Mikroskopie P 242, 5th edition, springer vertag, Berlin(1977)