ALKALINE PHOSPHATASE

(pNPP-DEA) KINETIC

BIO LAB DIAGNOSTICS
ISO 9001:2008
ISO 13485:2003
CE

CLINICAL SIGNIFICANCE

Elevation of Alkaline Phosphatase (ALP) level in the blood has clinical Indication of liver and bone diseases. Marginal Increase may indicate in the cases of congestive heart failure, Fanconl's syndrome, hyperparathyroidism, Hodgkin's disease and intestinal bacteriosis.

PRINCIPLE

$pNP + H_2O \xrightarrow{ALP}$ Nitrophenol + Pi

The rate of nitrophenol produced by the catalytic action of ALP is measured at 405 nM which is directly proportional to the quantity of alkaline phosphatase.

REAGENT COMPOSITION

pNP	10 mMol/L
Diethanolamine	1 Mo l/L
MgCl	0.5 mMol/L

PREPARATION OF WORKING REAGENT

Reconstitute pNP reagent R1 with Buffer Reagent R2 as per the volume indicated on the label of pNP reagent. Swirl to mix. The reagent is ready by 10 Minutes.

STABILITY

18 Months at 2-8°C. Reconstituted reagent is stable for 2 weeks at 2-8°C and 3 days at 25°C away from light.

SAMPLE

Sample can be serum or heparlnised plasma which has no sign of haemolysis. ***Avoid EDTA plasma.*** (All samples should be handled as potential Infective agents as no laboratory methods make conclusive findings for its safety. Therefore, adequate protective laboratory measures should be taken while handling such materials).

SYSTEM PARAMETERS

Reaction		Kinetic
Direction of Reaction		increasing
Temperature		37°C
Wavelength nM		405 (400-415)
Factor		5454
Absorbance Range		0-2°A
Cuvette Path Length		1 cM
Reagent Volume µL	500	1000
SampleVolume µL	5	10
Delay Time		60 seconds
Read Time		60 seconds
Max. limit of A / Minute		0.211
Dynamic Range		10-1150 IU/L
Max. limit of Blank Reagent		0.700

METHOD FOR DISCRETE ANALYSERS

For Cuvette Volume ..... µL	1000	500
Temperature ..... °C	37	37
Working Reagent ..... µL	1000	500
Sample ..... µL	10	5
Factor	5454	5454

Mix well. Take one minute reading at 405 nM after a delay of 60 seconds at 37°C.

NOTE: Programme the analyser using system parameters. A specific programme data sheet may be provided for each analyser upon request.

Reagent, sample volume and factor can be altered proportionately.

RESULTS Compute ΔA / Minute x Factor

EXPECTED VALUES

Temperature	30°C	37°C
Adults	50-232 IU/L	70-306 IU/L
Children below 15 years	140-488 IU/L	200-664 IU/L
Children around 16-17 years	100-366 IU/L	150-483 IU/L

As with all diagnostic methods, the final diagnosis should not be made on the result of a single test as well as laboratory diagnosis must be confirmed with clinical manifestations.

LIMITATIONS

This reagent system is altered when contaminated with Cu^{++}, Ag^{+}, Hg^{+}, EDTA leading to false results. pNP in solution deteriorates spontaneously therefore, each assay should be after a QC of the reagent. Discard the reagent which shows absorbance more than 1.0° A at 405 nM. ***This assay is linear to 1150 IU / L of alkaline phosphatase.***

QUALITY CONTROL

To ensure adequate quality control, each run should be accompanied with a standard quality control serum. The accuracy of the test must be established against the values of control serum calibrated as per this methodology. It should be realised that the use of quality control material checks both instrument and reagent functions together. Factors which might affect the performance of this assay include proper instrument functions, temperature control, cleanliness of glass wares, accuracy of volumetric systems, temperature control and timings.

WARNING

This reagent system is for *in vitro* use only.
This reagent system is containing preservatives and components that have not established for safety if contacted on broken skin or eye or taken orally. In case of such incident wash off with plenty of water, or consult a physician.

BIBLIOGRAPHY

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