ALKALINE PHOSPHATASE LYPHO

(Kinetic p-NPP with AMP Buffer)

Intended Use

Alkaline phosphatase is a reagent kit is used for the quantitative determination of Alkaline phosphatase activity in human serum or plasma based on kinetic method using p-nitrophenyl phosphate (p-NPP).

Method/ Principle

Kinetic method recommended by international federation of clinical chemistry (IFCC).

2-amino-2-methyl-1-propanol+p-nitrophenylophosphate+H2O ALP>4-nitrophenol+2-amino-2-methyl-1-propanol phosphate.

The rate of 4-nitrophenol formation is directly proportional of the ALP activity.

Reagents provided

1. R1 - Buffer solution
2. R2 - Substrate p-NPP Powder

Preparation of working solution

Transfer exact volume of buffer mentioned on the label of dry powder vial. Swirl the reagent till the powder gets dissolved completely. Keep it for 10 minutes to stabilize before use.

Reagent storage and stability

Prior to use:

When stored between 2-8°C the individual reagents are stable until the expiration date stated on the bottle and kit box label.

Working Reagent:

The working reagent is stable for 14 days when stored capped at 2-8°C or 2 days at room temperature (18-25°C).

Indications of Reagent Deterioration:

• Turbidity;
• Working Reagent absorbance < 0.900 Abs at 405nm
• Failure to recover control values within the assigned range.

Specimen collection and preservation

Blood should be collected in a clean and dry container. Haemolysed specimen should be avoided as it may falsely elevate results. EDTA, citrate and oxalate inhibit Alkaline Phosphatase activity and should not be used as anticoagulant.

For plasma separation any of the following two anticoagulants may be used:

- HEPARIN 200 IU/ml of blood
- SODIUM FLUORIDE 10 mg/ml of blood

Serum/plasma should be separated as quickly as possible from cells. Alkaline Phosphatase is stable for 4 days at 2° - 8°C and several months when stored at -20°C.

Assay guidelines for Analyzers

Reaction type – Slope	Kinetic – Increasing
Wavelength	405 nm
Flow Cell Temperature	37°C (± 0.2°C)
Zero setting with	Distilled Water
Delay time	60 seconds
Interval time	30 Seconds
No. of readings	3
Sample volume	25 µl (0.025 ml)
Reagent volume	1000 µl (1.0 ml)
Factor	2720
Low Normal	40 IU/L
High Normal	150 IU/L
Linearity	Up to 1200 IU/L

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Assay guidelines for Manual Procedure

Reagents	Test
Serum/plasma	25 µl (0.025 ml)
Working Reagent	1000 µl (1.0 ml)

1. Mix thoroughly and transfer the assay mixture immediately to the thermo stated cuvette and start the stop watch simultaneously.
2. Record the first reading at 60th second and subsequently, three more readings with 30 seconds interval at 405 nm.

Calculation

Calculate the average change in absorbance per minute.

Serum Alkaline Phosphatase (IU/L) = Δ Abs. / min x 2720

Normal Range

Female

1 to 12 Years	:	< 500 IU/L
> 15 Years	:	40 - 150 IU/L

Male

1 to 12 Years	:	< 500 IU/L
12 to 15 Years	:	< 750 IU/L
> 20 Years	:	40 - 150 IU/L

Note: Expected range varies from population to population and each laboratory should establish its own normal range.

Limitations

1. The working solution should not be used if the absorbance exceeds 0.900 at 405 nm against distilled water.
2. This method is linear up to 1200 IU/L. If the Δ Abs. / min exceed 0.250, dilute the sample (10 times) with normal saline and repeat the assay. Multiply the result obtained by 10.

Quality Control

To ensure adequate quality control measures, it is recommended that each batch should include a normal and an abnormal commercial reference control serum. It should be realized that the use of quality control material checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware and accuracy of pipetting.

References

1.IFCC methods for the measurement of catalytic concentration of enzymes. Part 5: IFCCmethods for alkaline phosphatase. *J Clin Chem Clin Biochem* 1983; 21: 731-748.

2. Young DS. Effects of drugs on clinical laboratory tests, 3th ed. AACC Press, 1997.

Symbols

IVD In Vitro Diagnostics. Caution. Keep away from sun light. Date of Manufacture.

LOT Batch No. Read Instructions. Fragile. Product Expiry Date.

CONT Content. Storage Temperature. Keep Dry. Manufactured By.

REF Catalogue No.

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