ALKALINE PHOSPHATASE

(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. Reagent (Ready to Use)

Reagent storage and stability

The reagent kit is stable till the expiry date stated on the label, when stored at 2° - 8°C.
This is critical because the reagent is light sensitive. It should therefore be kept away from direct light.

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	20 µl (0.02 ml)
Reagent volume	1000 µl (1.0 ml)
Factor	2720
Low Normal	53 IU/L
High Normal	128 IU/L
Linearity	Up to 1600 IU/L

Assay guidelines for Manual Procedure

Prewarm the required amount of working solution at the required temperature 37°C before use. Perform the assay as given below.

Reagents	**Test**
Serum/plasma	20 µl (0.02 ml)
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

Guidance value for Male	: 53 - 128 IU/L
Guidance value for Female	: 42 - 98 IU/L
Child	: 53 - 370 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 0.700 at 405 nm against distilled water.
2. This method is linear up to 1600 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. Bessey O.A., Lowry O.H. And Brock M.J.: Biol. Chem.,164,321,1946.
2. Bowers G.N. Jr. and McComb R.B., Clin. Chem. 12,70,1966.
3. McComb R.B and Bowers G.N.Jr. Clin. Chem.18,97,1972.
4. Z. Klin. Chem. Klin. Biochem.8, 658 (1970);9,464 (1971); 10,182 (1972).
5. Kubler W.: Symp. D. DeutschenGes. fur Lab. Med. Mainz (1973).

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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