ANA BIO CLR ALKALINE PHOSPHATASE

(Kinetic Method)

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).

Principle

Alkaline phosphatase cleaves p-nitrophenyl phosphate (p-NPP) into p-nitrophenol and phosphate. p-nitrophenol is a yellow colour compound in alkaline medium and absorbs light at 405 nm. The rate of increase in absorbance at 405 nm is proportional to Alkaline phosphatase activity in specimen.

p - nitrophenyl phosphate + H_2O $\xrightarrow{ALP}$ Phosphate + p – nitrophenol

Reagents provided

1. R1 - Diluent solution
2. R2 - Substrate p-NPP

Preparation of working solution

Prepare working reagent by mixing Reagent R_1 and Reagent R_2 in the ratio 4:1 as per the number of tests required.

Reagent storage and stability

The reagent kit is stable till the expiry date stated on the label, when stored at 2° - 8℃.
The working solution is stable for 15 days at 2° - 8℃.
The working solution should be prepared in the container provided and stored in the dark.
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℃ and several months when stored at -20℃.

Assay guidelines for Analyzers

Reaction type – Slope	Kinetic – Increasing
Wavelength	405 nm
Flow Cell Temperature	37℃ (± 0.2℃)
Zero setting with	Working solution
Delay time	60 seconds
Interval time	60 Seconds
No. of readings	3
Sample volume	20 µl (0.02 ml)
Reagent volume	1000 µl (1.0 ml)
Factor	2720
Low Normal	108 IU/L
High Normal	306 IU/L
Linearity	Up to 1000 IU/L

Assay guidelines for Manual Procedure

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

Reagents	**Test**
Serum/plasma	20 µl (0.02 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 60 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 Adult : 108 - 306 IU/L

Guidance value for Children : 210 - 810 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 1000 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.

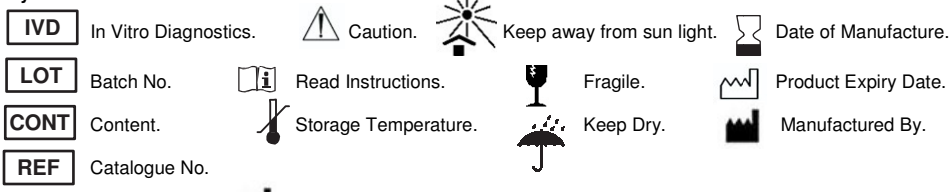
Quality Control

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. Hendry, R. J., "ENZYMES" in Clinical Chemistry Principle and techniques, Harper & Row Publishers, New Yark, 815 (1974).
2. Young, D.S. et al, Clin. Chem. 18, 1041, (1972).

Symbols



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