# Alkaline Phosphatase

## (p-NPP Kinetic Method)

Code : 11001/11002 (10x5 ml/2x50 ml)

(For the Analyser / Colorimetric Estimation of Triglycerides in Serum)

In VITRO USE Only.

## PRINCIPLE :

ALP activityhas been measured by many methods differing in substrate, buffer type, buffer concentration, time and temperature of incubation and unit of measurement. ALP KIT is based on the recommendations of the International Federation of Clinical Chemistry (I.F.C.C.). This method utilizes p-nitrophenylphosphate (p-NPP) as the substrate and the 2-amino-2-methyl-1-propanol buffer (AMP) ensure a high catalytic activity of ALP.

Serum ALP hydrolyzes p-NPP into yellow colored p-nitrophenol (p-NP) at an alkaline pH. The rate of p-NP formation is directly proportional to the ALP acitivity and is measured in terms of change in absorbance at 405nm.

$$\text{p-NPP} + H_2O \xrightarrow[Mg^{2+} \,/\, \text{alk pH}]{\text{ALP}} \text{P-NP} + \text{Phosphate}$$

## REAGENTS :

| | | | |
|---|---|---|---|
| 1. | p-NPP substrate | 10 x 5 ml | 2 x 50 ml |
| 2. | buffer Solution | 50 ml | 100 ml |

The reagents are ready to use and stable at 2-8$^{o}$C. till the expire date mentioned on the lable.

## REAGENTS PREPARATION :

Reconstitute one vial of p-NPP substrate (1) with 5 ml of buffer solution(2).

## SAMPLE :

1. Serum or heparinized plasma can be used.

2. Other anticoagulants such as EDTA. oxalate and citrate should not be used as they inhibit the enzyme activity by complexing $Mg^{2+}$ions.

| EXPECTED RANGE : | 30$^{o}$C | 37$^{o}$C |
|---|---|---|
| **Adults** | 30-90 U/L | 40-120 U/L |
| **Children upto 15 yrs.** | <250 U/L | <350 U/L |

## LINEARITY :

ALP KIT is Linear upto 900 U/L

## INSTRUCTIONS :

1. Avoid contamination of the reagent.
2. Sample should be assayed on the same day. if necessary they may be preserved upto 72 hours if frozen immediately.
3. Grossly haemolyzed samples should not be used.

## DIRECTIONS FOR USE ON ANALYSERS :

| | | |
|---|---|---|
| Reaction Type | : | Kinetic with factor |
| Wave Length | : | 405 nm |
| Incubation Temp | : | 30$^{o}$C/37$^{o}$C |
| Incubation Time | : | 30 Sec. |
| Rear Time | : | 180 Sec. |
| No. of Readings | : | 4 |
| Intervel Time | : | 60 Sec. |
| Sample Volume | : | 20 µl |
| Reagent Volume | : | 1 ml |
| Light path | : | 1 cm |
| Linearity | : | upto 9000U/L |
| Unit | : | U/L |
| Factor | : | 2742 |

## PROCEDURE :

1. Pipette into clean dry cuvette

| | |
|---|---|
| Reconstituted reagent | 1 ml |
| Bring to essay temperature & add sample | 0.02 ml |

2. Mixwell and read and record initial absorbance $A_o$ at 30 seconds and repeat reading after every minute for three minuts $A_3$

3. Calculate the absorbance change per minute.

$$DA/min. = \frac{A_3 - A_o}{3}$$

4. If the DA/minute is greater than 0.35 dilute one part of sample with 9 parts of isotonic saline and reassay. multiply the result by 10 to compensate for the dilution.

## CALCULATIONS :

Due to serum ALP activity in U/L = DA/min. x 2742

## NOTES :

★ Due to variations in inter - laboratory assay conditions, instruments and demography, it is recommended that each laboratory should establish its own normal range. To ensure adequate quality control, each run should include a normal and abnormal assayed controls. The assigned value of the control must be confirmed by this methodology.

★ Final diagnosis should be based on a co-relation of test results with other clinical observations / Diagnostic tools.

## BIBLIOGRAPHY :


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