

Alkaline Phosphatase

(p-NPP Kinetic Method)

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

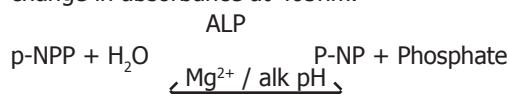
(For the Analyser / Colorimetric Estimation of Triglycerides in Serum)

In VITRO USE Only.

PRINCIPLE :

ALP activity has 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 activity and is measured in terms of change in absorbance at 405nm.



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°C. till the expire date mentioned on the label.

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°C	37°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°C/37°C
Incubation Time	:	30 Sec.
Rear Time	:	180 Sec.
No. of Readings	:	4
Interval 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 assay temperature & add sample	0.02 ml

2. Mix well and read and record initial absorbance A_0 at 30 seconds and repeat reading after every minute for three minutes A_3

3. Calculate the absorbance change per minute.

$$\text{DA/min.} = \frac{A_3 - A_0}{3}$$

4. If the DA/minute is greater than 0.35 dilute one part of sample with 9 parts of isotonic saline and re-assay. 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 :

1. Philip D Mayne "Plasma enzymes in Diagnosis in Clinical Chemistry in Diagnosis and treatment ELBS 1994 Chapter 15:299-313.
2. Tietz. N.W., Fundamentals of Clinical Chemistry, W.B. Saunders and Company, Philadelphia, PA (1976) 602.
3. Bowers, G.N. & McComb, R.B. (1972) Clin.Chem.18,97.

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