

INTENDED USE : In vitro test for the quantitative determination of alkaline phosphatase (ALP) in human serum.

PRINCIPLE :

The substrate p-nitrophenyl phosphate (pNPP) is hydrolysed by alkaline phosphatase present in the sample to p-nitrophenol (pNP) and phosphate in alkaline medium in presence of magnesium ions. pNP gives yellow colour. The intensity of colour corresponds to alkaline phosphatase activity and is read photometrically at 405 nm.



CLINICAL SIGNIFICANCE:

A physiological increase in alkaline phosphatase level due to active bone growth in children or in pregnancy from placental secretion is seen as a normal elevation. Increase in activity is seen in disease of bone, biliary tract and liver, certain drugs like androgens, anabolic steroids, estrogens, sulphonamides phenothiazine, tricyclic antidepressants, MAO inhibitors, antibiotics, leads to increase in alkaline phosphatase activity. Decreased activity seen in severe anemia, scurvy kwashiorkar and cretinism.

SPECIMEN COLLECTION AND STORAGE:

Fresh, clear and fasting unhemolysed serum is preferred. Anticoagulants like oxalates, citrates and EDTA should be avoided.

REAGENT STORAGE AND STABILITY

- When the reagent is stored properly at 2-8°C and the contamination is avoided, it is stable up to the expiry date mentioned on the labels and kit box.
- It is recommended that when the reagent is not in use, it should be strictly kept at 2-8°C for better performance and stability.
- Substrate Reagent must be protected from direct sun light.
- Do not expose the reagent to high temperatures above 10°C.
- ALP reagent should be discarded if the blank exceeds 1.2 at 405 nm.

SPECIMEN:

1. Unhemolysed serum is the preferred specimen. **DO NOT USE PLASMA**
2. Serum samples are stable for 24 hours at 2-8°C and for a month at -20°C. (Please note that ALP activity in serum stored at 2-8°C increase with time.)
3. Discard contaminated specimen.

REAGENTS:

ALP reagents are to be stored at 2-8° C

SYSTEM PARAMETERS:

| | |
|------------------------|-----------------|
| Reaction Type (Mode) : | Kinetic |
| Direction : | Increasing |
| Wave Length : | 405nm |
| Flow Cell Temp. : | 37°C |
| Zero Setting with : | Distilled Water |
| Delay time : | 60 seconds |
| Measuring Time : | 60 Seconds |
| No. of reading : | 2 |
| Reagent Volume : | 1ml |
| Sample Volume : | 25 µl |
| Factor : | 3200 |
| Linearity : | 1000 |
| Units : | IU/L |

TEST PROCEDURE:

Alkaline Phosphatase - SLR is a single liquid Reagent and is ready to use. *There is no reconstitution required.*

2 Minutes increasing kinetic reaction (60Sec Delay+60 Sec Measuring)

| | |
|-------------------|--------|
| Substrate Reagent | 1.0 ml |
| Serum | 25µl |

Mix well and aspirate into the analyzer. After 60 Sec incubation (Delay) measure the change of optical density during the next 60 seconds (Measuring Time) against distilled water at 405 nm as follows.

- Ao- Exactly after 60 Seconds.
- A1 Exactly after 60 seconds

CALCULATION

Calculate the average change in absorbance per minute ($\Delta A/\text{min}$) and multiply by the corresponding factor.

Activity of Alkaline Phosphatase in sample (IU/L):
 $= \Delta A/\text{min} \times 3200$

REFERENCE VALUES:

It is recommended that each Laboratory to establish its own reference values.

| | |
|---------|-----------------------|
| 20-50 Y | Males: 53 -165 U/L |
| | Females: 42 - 136 U/L |
| ≥60 Y | Males: 56 - 136 U/L |
| | Females: 53 - 160 U/L |

In growing children (Age ; 10-17 Years) the values will be Higher than adults i.e.350-585 IU/L.

SENSITIVITY :

The lower limit of detection is 2 IU/L

LINEARITY:

This method is linear for Alkaline Phosphatase activity up to 1000 IU/L. For sample values exceeding the linearity limit, dilute the sample suitably with normal saline and repeat the assay. Apply proper dilution factor while calculation.

BIBLIOGRAPHY:

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 RICK, W, Klinische chemie und Mikroskopie P. 242, 5th edition, Springer vertag, Berlin (1977).

Mfg. By: BIOGENIX INC. PVT. LTD.

Factory: B - 19/A, S.I.L Ancillary Estate, Amausi Industrial Area, Nadarganj, Kanpur Road, Lucknow - 226008 (U.P.), India

Email: biogenix2007@yahoo.com, info@biogenixinc.com

Website: www.biogenixinc.com

Customer care no: +919140971443

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