ALKALINE PHOSPHATESE

pNPP DEA Method

Intended Use:
Alkaline Phosphatase (ALP) or isozyme test reagent/kit is a medical device intended for the estimation of Alkaline Phosphatase or it isozyme in serum/plasma.

Clinical Significance
Serum Alkaline Phosphatase (ALP) measurements are of particular interest in the investigation of two groups of conditions: *bone disease* and *hepatobiliary disease*.
Among the bone diseases, the higher levels are found in Paget´s disease and in patients with osteogenic bone cancer, and moderate rises in osteomelacia and rickets, the latter falling to normal on treatment with vitamin D.
Physiological bone growth elevates Alkaline Phosphatase in serum of growing children and a transient elevation may be found during healing of bone fractures.
Causes of decreased plasma ALP level are: cretinism, vitamin D deficiency and hypophosphatasia, an hereditary bone disease.
The response to the liver to any form of biliary tree obstruction is to synthesize more Alkaline Phosphatase. Intrahepatic obstruction of the bile flow by invading cancer or drugs raises serum ALP. Any drug that is hepatotoxic or induces cholestasis will greatly increase serum Alkaline Phosphatase. Well over 200 drugs have been shown to increase serum ALP in susceptible patients.[4]

Principle
Alkaline phosphatase (ALP) catalyze the hydrolysis of 4-Nitrophenylphosphate (4-NPP) with the formation of free 4-Nitrophenol and inorganic phosphate, acting the alkaline buffer as a phosphate-group acceptor.
The reaction of monitored kinetically at 405nm activity by the of rate of formation of 4-nitrophenol, proportional to the activity of ALP present in the sample.

$$\text{4-NPP} + H_2O \xrightarrow[\text{Ph > g}]{\text{ALP, } Mg^{++}} \text{4-Nitrophenol} + P_i$$

REAGENT COMPOSITION

R1 ALP Reagent	pNPP 12mmol/L, Mg^2 10 mmol/L DEA buffer 8mmol/L

Working Reagent Preparation
Reagents are ready to use.
The reagent is light & temperature sensitive. Take adequate care, do not expose the reagent to direct light.

Stability and Storage
Store at 2-8ºC.
All the kit contents are stable until the expiry date stated on the label. Do not use reagents over the expiration date.
Store the vials tightly closed protected from light and prevent contaminations during the use.

Discard if signs of deterioration appear:
- Presence of particles and turbidity.
- Blank absorbance (A) at 405 nm > 1.000 in 1cm cuvette.

Materials required
- Photometer or spectrophotometer with a thermostat cell compartment set at 25/30/37ºC, capable of reading at 405 nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm path length
- Pipettes to measure reagent and samples.

Sample and Stability

Serum or plasma
Serum or heparinized plasma free of hemolysis. Other anticoagulants such as EDTA, oxalate and citrate inhibit the enzyme by complexing Mg^{++} and should not be used. Alkaline phosphatase in serum or plasma is stable for 7 days at 2-8ºC.

Known Interfering Substances
- Lipemia (intralipid 20 g/L) do not interfere.
- Bilirubin (20 mg/dL) does not interfere.
- Hemoglobin (>2 g/L) may affect the results.
- Other drugs and substances may interfere[2,3].

Assay Procedure

1. Pre-incubate working reagent, samples and controls to reaction temperature
2. Set the photometer to 0 absorbance with distilled water.
3. Pipette into a cuvette:

R1 ALP (MR) Reagent	1000 µl
Sample or control	25 µl

4. Mix gently by inversion. Insert cuvette into the cell holder and start stopwatch
5. Incubate for 1 minute and record initial absorbance reading.
6. Repeat the absorbance readings exactly after 1, 2 and 3 minutes.
7. Calculate the difference between absorbance.
8. Calculate the mean of the results to obtain the average change in absorbance per minute (ΔA/min).

Calculation
ALP ACTIVITY (IU/L) = ΔA/min x 2754 x tf

Samples with ΔA/min exceeding 0.250 at 405 nm should be diluted 1:2 with saline and assayed again. Multiply the results by 2.

If results are to be expressed as SI units apply:
IU/L x 0.01667 = µkat/L

Temperature Conversion Factor

Assay Temperature	Factor (tf)
25^0C	1.7
30^0C	1.34
37^0C	1.00

Quality control
To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

If the values are found outside of the defined range, check the instrument, reagents and procedure.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable results.

Reference Values

Serum (Adults) : 80-290 IU/L at 37^{0}C

(Children) : 245-770 IU/L at 37^{0}C

It is recommended that each laboratory establishes its own reference range.

General System Parameters

Mode	Kinetic
Reaction	Increasing
Wavelength	405 nm
Blank with	Distilled water
Sample Volume	25 µL
Reagent Volume	1000 µL
Delay Time	60 sec
Measuring Time	180 sec
Interval time	60 sec
No. of readings	3
Factor	2754
Linearity limit	Up to 1000
Unit	IU/L

Linearity

The procedure is linear up to 1000 IU/L at 37ºC. If the absorbance change (ΔA/min) exceeds 0.250, use only the value of the first two minutes to calculate the results, or dilute the sample 1+9 with normal saline (Nacl 0.9%) and repeat the assay (Results x 10).

Note:

1. Samples having a very high activity show a very initial absorbance. If this is suspected then dilute the sample and repeat the assay. Adherence to the reaction time should be meticulously followed. Substrate Reagent and Working Reagent should not be exposed to light or left at R.T.
2. To avoid contamination it is recommended to use new tip for dispensing the reagent.
3. If the blank exceeds 3.0 OD, discard the solution.
4. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

Reference

1. German Society for Clinical Chemistry: Recommendations of the Enzyme Commision. Z. Klin. Chem. Klin. Biochem. 10 : 281 (1972).
2. Young, D.S. Effects of Drugs on Clinical Laboratory Tests. 4th Edition. AACC Press (1995).
3. Tietz. N.W. Clinical Guide to Laboratory Tests, 3rd Edition. W.B. Saunders Co. Philadelphia, PA. (1995).
4. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.

For *in vitro* Diagnostic use only.