Intended Use
For **IN VITRO quantitative** determination of Alkaline Phosphatase in serum using manual or automated applications.

Clinical Significance
Measurements of ALP activity are used for diagnosing liver, bone, and intestinal syndromes, as well as for monitoring the causes and treatments. (1)

Method History
In 1930, Kay published a method for Alkaline Phosphatase (EC 3.1.3.1). (2) Since then, various modifications have been proposed and used. Bessey, Lowry and Brock (3) introduced p-nitrophenyl phosphate (PNPP) as a more sensitive substrate. Catachem's ALP method is based upon the work of Bessey, et al.

Method Principle
Alkaline Phosphatase catalyzes the conversion of p-nitrophenyl phosphate (PNPP) to p-nitrophenol. P-nitrophenol is a bright yellow-colored compound which has maximum absorbance at 405nm. The rate of increase in absorbance from p-nitrophenyl phosphate (colorless) to p-nitrophenol (color) is directly proportional to the AP enzyme activity in the serum sample. The reaction scheme illustrates the reaction that occurs in this method.

$$\text{PNPP} + H_3P0_4 + H_20 \xrightarrow[Mg^{++}]{\text{ALP}} \text{P-nitrophenol}$$

Reagent Content
When reconstituted according to the directions, the concentrations of the active ingredients in the reagents will be approximately as follows:

PNPP Diluent
Each liter contains:
Buffer
Nonreactive ingredients

PNPP Reagent
Each vial contains:
PNPP 10.8 mmol/L

Precautions
Avoid Contact of reagent with skin and eyes. Should contact occur, wash affected area with plenty of cold water. **DO NOT PIPETTE REAGENTS BY MOUTH.**

Preparation Of Working Reagent
Prepare the required number of vials of Catachem ALP Working Reagent by adding the appropriate amount of diluent (as noted on package insert) with PNPP substrate reagent. Mix well until completely in solution.

Reagent Storage And Stability
Store the Catachem ALP Reagents at 2-8˚C. When stored as directed, the reagents are stable until the expiration date stated on the label. When prepared and stored as directed, the Working PNPP Reagent is stable for at least fifteen days at 2-8˚C.

Specimen Collection And Preparation
Fresh, clear, unhemolyzed samples should be collected. Anticoagulants inhibit the action of alkaline phosphatase, therefore, anticoagulants should be avoided.

Precautions
Avoid contact of specimen with skin and eyes. Should contact occur, wash affected area with plenty of water. **DO NOT PIPETTE SPECIMENS BY MOUTH.**

Quality Control
To monitor the performance of the Working Reagents and the procedure used, we recommend the regular use of normal and abnormal control serum.

Interfering Substances
The following substances, if present in the sample, reagents or system, can produce erroneous results: arsenic, beryllium salts, fluorides, bromisulfalein, manganese salts. For a more comprehensive discussion on the effect of interfering substances on various AP methods, including the PNPP method, refer to the cited literature. (4)

Expected Values
The range of expected values determined for this method is 30 U/L to 115U/L for human samples. These values are suggested guidelines. It is recommended each laboratory establish the normal range for the species under study and for the area in which the laboratory is located.

Procedure

Important: Read the entire procedural instructions before proceeding with the assay.

Materials Required (But Not Provided)

Spectrophotometer
Matched cuvettes 1 cm light path

Timer	to time incubation time
Pipette	1.0 ml for reagent
Pipette	0.03 ml for sample
Graduated cylinder	25 ml or 50 ml for reagent

Materials Provided

Catachem Alkaline Phosphatase Reagent

Analytical Parameters

Wavelength	405 nm
Temperature	37˚C
Path length	1 cm
Reaction Mode	rate
Reaction Time	5 minutes
Reagent Volume	1.0 ml
Sample Volume	0.03 ml
Total Volume	1.03 ml
Sample-to-reagent ratio	1:34

Assay Procedure

1. Set spectrophotometer wavelength at 405nm and zero the instrument with the cuvette containing water.
2. Pipette 1.0 ml of Working Reagent into each of three cuvettes marked “Sample” and “Control”.
3. Incubate cuvettes for 2 minutes at 37˚C.
4. Pipette 0.03 ml of Control and Sample into their respective cuvettes. Mix all cuvettes well.
5. Replace the cuvettes in spectrophotometer and continuously monitor the change in absorbance for at least 5 minutes.
6. Read the “Control” and “Sample” absorbencies.
7. Calculate the ALP concentration (u/L) in the sample(s), as shown in results and calculations.

Results And Calculations

$$\text{ALP (u/L)} = \frac{\text{Delta A/min} \times 1.03 \text{ ml} \times 1000}{18.9 \times \text{L(cm)} \times \text{Sample volume}}$$

Where:

Delta A/min	= change in absorbance per minute
1.03 ml	= total volume in cuvette
0.03 ml	= volume of sample being assayed
18.9	= Extinction coefficient
1000	= converts U/ml to U/L

Example: Delta A/min = 0.01

$$\text{ALP (u/L)} = \frac{0.01}{\text{min}} \times \frac{1}{18.9} \times \frac{1.03 \text{ ml}}{0.03 \text{ ml}} \times 1000$$

$$= 18 \text{ u/L}$$

Method Performance Characteristics

Sensitivity: The sensitivity of this method is 0.00080-0.0010 absorbance units per U/L.

Linear Range: In this method there is no significant nonlinearity over the range of 0-1200 u/L.

Precision: Within-run and day-to-day precision is summarized below.

Precision Study

ALP	Within-Run Precision		Total Precision	
Mean	SD	CV	SD	CV
U/L	U/L	%	U/L	%
49	1.48	3.00	3.80	7.80
261	0.71	0.27	2.20	0.80
500	2.10	0.40	6.50	1.30

Correlation

A comparison of this method using an automated analyzer and a reference method resulted in the following regression statistics.

Range	=	30-665
N	=	153
Y	=	0.948x + 34
r	=	0.999
Sy.x	=	4.7

References

1. Fundamentals of Clinical Chemistry. Edited by Norbert W. Tietz. (1976) W.B. Saunders Company, Philadelphia.
2. Kay H.D. Plasma Phosphatase 1: Method Determination. J. Biol Chem 89, 235 (1903).
3. Bessey O.A., Lowry S.H., Brock M.H. A method for the rapid determination of alkaline phosphatase with five cubic milliliters of serum. J Biol Chem 164, 321 (1946).
4. Young D.S., Pestaner L.C., Gibberman V. Effects of drugs on clinical laboratory tests. Clin Chem 21 No. 5(1975).

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