ALKALINE PHOSPHATASE (ALP) REAGENT KIT C174-0D

Contents	Product No.	Package
ALP KIT	**C174-0D**	
ALP Sample Diluent	C174-07	6 x 45 mL
ALP Activator	C174-08	6 x 5.0 mL

WORKING REAGENT PREPARATION

Transfer the contents of one ALP Activator Reagent vial into bottle of ALP Sample Diluent Reagent.
If necessary, rinse Activator vial, with combined Sample Diluent Reagent.
Mix the combined reagent in Sample Diluent Reagent vial by inverting bottle several times.

REAGENT STORAGE AND STABILITY

Store the unopened reagent kit at 2-8°C.
When stored as directed, both unopened reagents are stable until the expiration date stated on the label.

NOT FOR USE IN UNPROFESSIONAL SETTINGS

FOR TECHNICAL ASSISTANCE:
Email: catachem@catacheminc.com
Contact Form: www.catacheminc.com
Call: 203-262-0330

ALKALINE PHOSPHATASE (ALP) REAGENT KIT C174-0D MANUAL/AUTOMATED APPLICATION

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 405 nm. 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.

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

Reagent Content
The reagent is comprised of two liquid stable reagents R1 and R2.

R1 Reagent
Each liter contains:
Buffer
Nonreactive ingredients

R2 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.**

Reagent Preparation for Automated Analyzers
Catachem ALP reagents are supplied ready-to-use on most automated platforms as dual reagents. If you require a freshly prepared single working reagent, add 1 part of R2 to 9 parts of R1.

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 opened each reagent is stable for at least 30 days (capped when not in use) at 2-8˚C. When prepared as a single reagent the Working Reagent is stable for at least fifteen days at 2-8˚C. The Catachem ALP Reagents have been tested to reflect extreme shipping conditions and are stable for the lifespan of the product if frozen up to 5 times or upon reaching temperatures up to 40°C for up to one week.

Specimen Collection and Preparation
Fresh, clear, non-hemolyzed samples should be collected. Anticoagulants inhibit the action of alkaline phosphatase; therefore, anticoagulants should be avoided. Catachem recommends Plain (red top) or SST (tiger top) tubes for sera collection.

Quality Control
To ensure optimal performance of these reagents and this procedure, assay performance should be monitored by running normal/abnormal controls concomitantly with samples. Catachem has optimized this assay using Catatrol Level I (C1200-11) and Catatrol Level II (C1200-12) controls and recommends their use for daily QC.

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

Expected Values
The analytical measuring range of this assay, as performed below, is 30 U/L to 115 U/L. These values serve as suggested reference points only. For veterinary samples ranges will be dependent on the species under test. It is recommended that each laboratory establish the normal ranges for the species under study and for the geographic 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 capable of reads @ 405nm
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, if used manually as a single working reagent.

Wavelength	405 nm
Temperature	37˚C
Path length	1 cm
Reaction Mode	rate

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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 a cuvette containing water.
2. Pipette 1.0 ml of Working Reagent into a minimum of two cuvettes marked “Sample” and “Control”. (More if needed for additional samples.)
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. Monitor the average change per minute (Delta A/min) of 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 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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