Alkaline Phosphatase LiquiColor®
Procedure No. FT677

Intended Use
For the Kinetic Quantitative Determination of Alkaline Phosphatase in Serum for Manual and/or Automated Procedures

Summary and Principle
Serum Alkaline Phosphatase (ALP) levels are of interest in the diagnosis of hepatobiliary disorders and bone disease associated with increased osteoblastic activity. Only slight to moderate elevations occur in osteomalacia, rickets, and Fanconi's syndrome. Serum enzyme activities may reach 10 to 12 times the upper limit in hepatic obstruction and return to normal after surgical removal. The sera of growing children and women in the third trimester of pregnancy also show increased levels of ALP activity.[1]

Serum ALP activity can be measured using various phosphate esters as substrates .[2] Alkaline Phosphatase procedure measures serum ALP activity by a kinetic method similar to the procedure described by Bowers and McComb using 4-nitrophenyl phosphate as substrate.[3]

$$\text{4-Nitrophenyl Phosphate} + H_2O \xrightarrow{\text{ALP}} \text{4-Nitrophenol} + \text{Phosphate}$$

Alkaline Phosphatase hydrolyzes 4-nitrophenyl phosphate to form 4-nitrophenol and phosphates. 4-nitrophenol is yellow in color, at pH 10.4 with an absorbance peak at 405 nm. The rate at which 4-nitrophenol is formed is directly proportional to alkaline phosphatase activity.

Reagent
Alkaline Phosphatase Buffer (R1), Cat. No. FT677a/d

Composition:

2-Amino-2-methyl-1-propanol, pH 10.4	0.35 mol/L
Magnesium Chloride	2.0 mmol/L
Zinc Sulfate	1.0 mmol/L
HEDTA	2.0 mmol/L

Alkaline Phosphatase Substrate (R2), Cat. No. FT677b

Composition:

4-Nitrophenyl Phosphate	16 mmol/L

Precautions: The reagents are for "In Vitro Diagnostic Use". Normal precautions exercised in handling laboratory reagents should be followed. The reagents contain sodium azide which may be toxic if ingested. Sodium azide may also react with lead and copper plumbing to form highly explosive metal azides. Refer to Material Safety Data Sheet for any updated risk, hazard or safety information.

Reagent Preparation: Buffer and Substrate liquid reagents are supplied ready-to-use. Prepare Working Reagent in the ratio of 5 parts Buffer (R1) to 1 part Substrate (R2) (i.e., 25 mL Buffer and 5 mL Substrate).

Reagent Storage and Stability: Reagents are stable until the expiration date on their respective labels, when properly stored at 2-8°C and protected from light. R1 should appear clear/colorless while R2 should appear clear/yellow. Discard if either appears cloudy or contains particulate matter. The Working Reagent is stable for 4 weeks at 2-8°C or 5 days at room temperature (15-30°C).

Material Required But Not Provided
Spectrophotometer capable of absorbance reading at 405 nm and 1 cm lightpath
Constant temperature block or bath, 37°C, or temperature controlled cuvet
Accurate pipetting devices
Test tubes
Interval timer

Specimen Collection and Storage
Serum or heparinized plasma, free of hemolysis, should be used. Complexing anticoagulants such as citrate, oxalate, fluoride and EDTA must be avoided.[4] Serum ALP is relatively stable for 7 days, if the sample is refrigerated (2-8ºC). However, on storage the enzyme activity increases slightly.[4] This increment in ALP activity is also observed with some reconstituted control sera, stored both at room temperature and in the refrigerator.[5] Bilirubin levels up to 40 mg/dL and triglyceride levels up to 2000 mg/dL show no interference in this test.[7]

Interfering Substances: EDTA, citrate, and oxalate inhibit ALP activity.[6] Certain drugs and other substances are also known to affect ALP values.[4]

Automated Procedure
Special adaptations for automated analyzers are available by contacting Interchim's Customer Service Department.

Manual Procedure
1. Prepare Alkaline Phosphatase Working Reagent according to instructions.
2. Zero spectrophotometer at 405 nm with distilled water.
3. For each sample and control, add 1.0 mL Working Reagent to cuvet or test tube and warm to 37°C for 3 minutes.
4. Add 20 µL (0.020 mL) serum to its respective tube and mix gently.
5. Read and record absorbance at 1 minute. Continue incubating at 37°C and record absorbance again at 2 and 3 minutes. Rate should be constant.

Determine the average absorbance per minute (A/min), multiply by factor 2764 for results in U/L.

NOTE: If cuvet is not temperature controlled, incubate samples at 37°C between readings.

Quality Control: Ser-T-Fy® I, Normal Control Serum, Cat. No. FT7670 and Ser-T-Fy® II, Abnormal Control Serum, Cat. No. FT7680 are recommended for verifying accuracy and precision. Other commercially available controls with ALP values assayed by this method are also suitable. ALP activity determined in these materials, by this procedure should fall within the ranges stated for the controls. Two levels of controls should be analyzed each day of testing.

Calibration: ALP activity is based on the " micromolar extinction coeffi-cient" of 4-nitrophenol at 405 nm (see "Results" section). The instrument manufacturer's calibration guidelines should be followed to calibrate your analyzer.

Results
Values are derived based on the "absorptivity micromolar extinction coefficient" of 4-nitrophenol at 405 nm (0.01845). Units per liter (U/L) of Alkaline Phosphatase activity is that amount of enzyme which products one mmol/L of 4-nitrophenol per minute.

$$U/L = \frac{\Delta A / Min}{Absorptivity} \times \frac{Total\ Volume}{Sample\ Volume}$$

$$U/L = \frac{\Delta A / Min}{0.01845} \times \frac{1.020}{0.020}$$

U/L = A/Min x 2764

Limitations
If the A/min. is greater than .250, dilute 1 part sample with 9 parts isotonic saline and re-assay. Multiply results by 10.

Expected Values[1]
Normal Range (Adult): 34 - 114 U/L (37°C)

This range should serve only as a guideline. It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments, laboratories, and local populations.

Performance Characteristics
Comparison: A group of 63 sera ranging in ALP activity from 0 - 1311 U/L was assayed by the described ALP method and by a similar commercially available ALP reagent. Comparison of the results yielded a correlation coefficient of 0.999 and the regression equation was y = 0.934x - 3.24. (Comparison studies were performed according to NCCLS Tentative Guideline, EP9-T.)

Precision: Within-run precision was established by 20 assays on three different levels of commercial serum controls. Total Precision values were obtained by assaying the 3 commercial controls for 5 consecutive days.

Within-Run

	Serum 1	Serum 2	Serum 3
Mean ALP (U/L)	139	262	327
Std. Deviation (U/L)	2.4	3.1	3.0
C.V. (%)	1.7	1.2	0.9

Total Precision

	Serum 1	Serum 2	Serum 3
Mean ALP (U/L)	146	258	323
Std. Deviation (U/L)	2.6	2.7	4.0
C.V. (%)	1.8	1.0	1.3

Precision studies were performed according to NCCLS Tentative Guideline, EP5-T.

Linearity: Linear to 800 U/L at 37°C.[7] Performed according to NCCLS Guideline EP6-P.

Sensitivity: Based on an instrument resolution of A = 0.001, the method presented shows a sensitivity of 2.0 U/L.

References

1.Tietz Textbook of Clinical Chemistry, 2nd ed. W.B. Saunders Co., Philadelphia, PA, p. 831-832 (1994)
2.Fujita H: Uber die microbestimmung der Blut Phosphatase. J. Biochem (Japan) 30: 69, 1939
3.Bowers, G.N., Jr., McComb, R.B.: A continuous spectrophotometric method for measuring the activity of serum alk. phos. Clin Chem 12:70-89, 1966.
4.Young, D.S., Pestaner, L.C., Gibberman, V.: Effects of drugs on clinical laboratory tests. Clin Chem 21: 246D, 1975
5.Massion, C.G., Grankenfeld, J.K.: Alkaline phosphatase: Liability in fresh and frozen human serum and in lyophilized control material. Clin Chem 18: 366, 1972.
6.Demetriou JA, Drewes DA, Gin JB: Enzymes. In Clinical Chemistry - Principles and Technics 2nd ed. RJ Henry, DC Cannon, JW Winkelman, Eds. Harper & Row, Hagerstown MD, 1974, p 927.
7.Interchim Laboratory Data

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