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

Material Required But Not Provided
Spectrophotometer capable of absorbance reading at 405 nm and 1 cm pathlength
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, and EDTA must be avoided.4 Serum ALP is relatively stable for 7 days, if the sample is refrigerated (2-8°C). However, on storage the enzyme activity increases slightly.3 This increment in ALP activity is also observed with some reconstituted control sera, stored both at room temperature and in the refrigerator.3 Bilirubin levels up to 40 mg/dL and triglyceride levels up to 2000 mg/dL show no interference in this test.6

Interfering Substances: EDTA, citrate, and oxalate inhibit ALP activity.5 Certain drugs and other substances are also known to affect ALP values.3

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 coefficient" 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 produces one mmol/L of 4-nitrophenol per minute.

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\frac{\text{U/L}}{\text{Absorptivity}} = \frac{\Delta A / \text{Min}}{\text{Sample Volume}} \times \frac{\text{Total Volume}}{\text{U/L}}
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<thead>
<tr>
<th>U/L</th>
<th>Absorptivity</th>
<th>Total Volume</th>
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<tbody>
<tr>
<td>0.01845</td>
<td>1.020</td>
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\[
U/L = A/\text{Min} \times 2764
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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
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, EP6-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.

Safety Information
Read labels and Material Safety Data Sheet for any updated risk, caution or safety information.

Reference
2. Fujita H, Ober die microbestimmung der Blut Phosphatase. J. Biochem (Japan) 36: 69, 1939
7. Interchim Laboratory Data