

Alkaline Phosphate

1.0 INTENDED USE

This reagent is intended for the quantitative determination of Alkaline Phosphatase in serum.

2.0 BACKGROUND

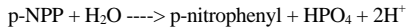
2.1 METHOD AND HISTORY

Alkaline Phosphatase (AP) activity was first measured by Kay (10.1). Since that time many substrates such as glycerol phosphate and phenyl phosphate have been used. Bessey, Lowry, and Brock (10.2) introduced a more sensitive substrate p-nitrophenyl phosphate (p-NPP). McComb and Bowers (10.3,10.4) studied the optimum buffer conditions for measuring AP activity in human serum. This procedure is based on the recommendations of Bowers and McComb.

2.2 TEST PRINCIPLE

The alkaline phosphatase hydrolyzes p-NPP to form the yellow chromogen p-nitrophenyl according to the following equation.

AP



The rate of increase in absorbance of the reaction mixture due to the formation of p-nitrophenyl is proportional to the alkaline phosphatase activity.

2.3 CLINICAL SIGNIFICANCE

Increased rates of bone syntheses cause elevated levels of alkaline phosphatase activity. Children who are still undergoing skeletal growth have up to three times the activity of a normal adult. The highest levels from increased osteoblastic activity occur in Paget's disease. Rickets also result in increased activity.

Placental alkaline phosphatase causes an increased serum level of the enzyme during the third trimester of pregnancy. A declining level at this time is indicative of placental insufficiency.

Hepatic alkaline phosphatase is normally produced by the biliary epithelium and passes down the biliary tree into the gut. Obviously, hepatic obstruction would cause levels to elevate.

3.0 SPECIMEN COLLECTION AND HANDLING

3.1 PATIENT PREPARATION

No special patient preparation is required.

3.2 SPECIMEN COLLECTION.

Fresh, clear, unhemolyzed serum is the preferred specimen. EDTA, Oxalate and citrate inhibit the action of alkaline phosphatase. Therefore these anticoagulants should be avoided.

Use a standard venipuncture tube to draw patient sample.

The amount of sample required will depend on the analyzer used. The amount of serum required is in the range of 5-25 μl . Call Biotrons technical service department at 1-800-595 8766 for the recommended sample volume for your analyzer.

Record the patient's name, date and time of sample collection and preparation.

3.3 SPECIMEN STORAGE

Serum for alkaline phosphatase assay may be stored at room temperature (18-26° C) for up to 8 hours. Samples are stable for 4-5 days at 2-8° C and for several months at -10° C. However, it has been reported that increased activities are found after storage (10.8.)

It is recommended that testing be done as soon as possible after sample collection and preparation. If testing cannot occur immediately, store the sample properly using the guidelines above.

4.0 MATERIALS

(10 X 10 ml)
(6 X 50 ml)

Reagents necessary for the determination of alkaline phosphatase are included in the kit.

4.1 REAGENT

Alkaline Phosphatase reagent contains:

magnesium acetate $\geq 3.0 \text{ mM/L}$
p-nitrophenyl phosphate $\geq 11.0 \text{ mM/L}$

Alkaline Phosphatase buffer contains

AMP buffer $\geq 0.3 \text{ mM/L}$

4.2 WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use. Not for Internal use in Humans or Animals. In Vitro Diagnostics reagents may be hazardous. Avoid ingestion and skin or eye contact.

4.3 REAGENT PREPARATION

4.3.1

Reconstitute each vial with 10 ml of alkaline phosphatase buffer. Replace the rubber stopper and allow 5 minutes for reconstitution. Swirl gently until the contents of the vial are completely dissolved. Record the date and time of reconstitution.

4.3.2

Reconstitute each vial with 50 ml of alkaline phosphatase buffer. Replace the rubber stopper and allow 5 minutes for reconstitution. Swirl gently until the contents of the vial are completely dissolved. Record the date and time of reconstitution.

4.4 REAGENT STORAGE AND STABILITY

When stored at 2°-8°C unopened reagents are stable until the expiration date printed on the label. Reconstituted reagent is stable for 30 days at 2°-8°C or 24 hours at 18°-26°C.

4.5 ADDITIONAL MATERIALS REQUIRED

4.5.1 A spectrophotometer or colorimeter capable of reading absorbance accurately at 405 nm.

4.5.2 1 cm cuvettes or a flow cell capable of transmitting light at 405 nm.

4.5.3 Test tubes capable of holding 3 ml.

4.5.4 Pipettes capable of delivering 2.5 ml and 25 μl .

4.5.5 Timer with one minute increments.

4.5.6 Constant temperature heat source which can be adjusted to 30° C or 37° C.

4.5.7 Normal and abnormal control for quality control.

5.0 TEST PROCEDURE

The following is a general procedure for use on a manual instrument.

5.1 PROCEDURE CONDITIONS

Wavelength	405 nm
Temperature	30° C or 37° C
Pathlength	1 cm
Mode	Kinetic
Reaction Time	2 - 4 min.
Sample Volume	25 μl
Reagent volume	2.5 ml
Total Volume	2.525 ml
Sample to reagent ratio	1/100

5.2 INSTRUMENT

Any instrument capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 405 nm may be used. The band width should be 10 nm or less, stray light 0.5% or less, and the wavelength accuracy within 2 nm.

5.3 CALIBRATION

No reagent calibration is necessary as the alkaline phosphatase activity is calculated by use of the molar absorptivity of p-nitrophenyl which is taken as 18.8 at 405nm.

5.4 PROCEDURE

5.4.1 Prepare the required number of alkaline phosphatase working reagent. (See 4.3 Reagent Preparation section.)

5.4.2 Into separate test tubes pipette 25 µl of serum to be assayed.

5.4.3 Add 2.5 ml of working reagent mix and incubate for two minutes at 30° C or 37° C.

5.4.4 Record the absorbance at one minute intervals until the absorbance change is constant.

5.5 CALCULATION AND RESULTS

Alkaline Phosphatase U/L =

$\Delta A/\text{min} \times \text{assay volume (ml)} \times 1000$

----- = $\Delta A/\text{min} \times 5372.3$

$18.8 \times \text{light path (cm)} \times \text{sample volume (ml)}$

$\Delta A/\text{min}$ = change in absorbance per minute

assay volume = total reaction volume expressed in ml

1000 = converts U/ml to U/L

18.8 = absorbance coefficient of p-nitrophenyl at 405 nm

lightpath = length of the light path expressed in cm (usually 1)

sample volume = sample volume expressed in ml

5372.3 = factor derived from constants in the equation

Example:

Alkaline Phosphatase U/L =

$0.019 \times 2.525 \times 1000$

----- = $0.019 \times 5372.3 = 102 \text{ U/L}$

$18.8 \times 1 \times 0.025$

0.019 = change in absorbance per minute

2.525 = total reaction volume in ml

1000 = converts U/ml to U/L

18.8 = absorbance coefficient of p-nitrophenyl at 405 nm

1 = light path in cm

0.025 = sample volume in ml

6.0 INTERPRETATION OF RESULTS

6.1 EXPECTED VALUES (10.4)

The range of expected values is:

25 - 90 U/L (30° C)

33 - 120 U/L (37° C)

These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

6.2 MEDICAL ALERT VALUES (10.9)

Each laboratory should establish low and high values beyond which the patient would require immediate attention by a physician. If a "medical alert value" is reached, always repeat the test to confirm the result and notify a physician if the result is confirmed.

6.3 LIMITATIONS OF PROCEDURE

A number of substances have been reported to cause physiological changes in serum alkaline phosphatase concentrations. (10.5-10.7)

As with any chemical reaction, users should be alert to the possible effect on results caused by unknown interferences from medications or endogenous substances. All patient results should be evaluated in light of the total clinical status of the patient.

7.0 QUALITY CONTROL

Standard practice for quality control should be applied to this system. Commercially available lyophilized controls can be used to monitor the daily acceptable variations. Normal and abnormal controls should be assayed at the beginning of each run of patient samples, whenever a new reagent or a different lot number is being used, and following any system maintenance.

A satisfactory level of performance is achieved when the analyte values obtained are within the "acceptable range" established by the laboratory.

8.0 CALIBRATION PROCEDURES

No reagent calibration is necessary as the alkaline phosphatase activity is calculated by use of the molar absorptivity of p-nitrophenyl which is taken as 18.8 at 405nm.

9.0 PERFORMANCE CHARACTERISTICS

9.1 PRECISION

The estimates of precision shown below were obtained from assays of human control serum.

Within-Run: In this study, 15 replicates of 2 control sera were run.

Mean (U/L)	SD (U/L)	CV (%)
------------	----------	--------

111	± 0.50	0.45
-----	--------	------

275	± 1.35	0.49
-----	--------	------

Between-Run: In this study, 5 runs were made, each run consisting of 5 replicates of 2 control sera.

Mean (U/L)	SD (U/L)	CV (%)
------------	----------	--------

86	± 0.73	0.85
----	--------	------

290	± 2.11	0.73
-----	--------	------

9.2 CORRELATION

A correlation study was done on the Technicon RA-500 system at 37° C comparing this method and a similar alkaline phosphatase method. The samples range between 44 and 447 U/L.

Number of Samples	Regression Equation $y = \text{Biotron}, x = \text{Comparative}$	Correlation Coefficient
28	$y = .937x + 14.9$	0.990

9.3 LINEARITY

This procedure is linear through 1000 U/L beyond which the specimen should be diluted with an equal volume of deionized water. Reassay the specimen and multiply the results by 2.

9.4 SENSITIVITY

The average sensitivity for this method is 0.0002 $\Delta A/\text{min}$ per unit of concentration (U/L).

10.0 REFERENCES

10.1 Kay, H.D., J. Biol Chem. 89,235(1930)

10.2 Bessey, O.A., Lowry, S.H. Brock, M.H., J. Biol. Chem. 164,321(1946)

10.3 McComb, R.B., Bowers, G.N., Clin. Chem. 18,97(1972)

10.4 Bowers, G.N., McComb, R.B., Clin. Chem. 21, 1988-1995(1975)

10.5 Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 3rd ed., Washington DC, AACC Press (1990).

10.6 Martin, E.W., Hazard of Medication, Philadelphia, PA and Toronto, Canada, J.B. Lippencott Company (1971) pp 169-189

10.7 Contantino, N.V. and Kabat H.F., Drug-induced modifications of laboratory test values, revised 1973, Am J Hosp Pharm 30:24-71 (1973)

10.8 Bowers, G.N., McComb, R.B., Clin. Chem. 12,70(1966)

10.9 G.J. Kost, "Critical Limits for Urgent Clinician Notification at U.S. Medical Centers"; JAMA, Feb. 2, 1990; Vol 263, No.5, p.704