

Alkaline Phosphatase (Liquid)

1.0 INTENDED USE

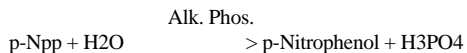
This reagent is intended for the quantitative determination of Alkaline Phosphatase in serum.

2.0 BACKGROUND

2.1 METHOD AND HISTORY

Alkaline phosphatase in serum is determined by measuring the rate of hydrolysis of various phosphate esters under specified conditions. P-Nitrophenyl Phosphate is one such phosphate ester and was introduced as a substrate by Fujita in 1939. (10.2). Bessey, Lowry, and Brock published an endpoint procedure in 1946 (10.3) while Bowers and McComb reported a kinetic procedure in 1966 (10.4). The kinetic procedure has undergone several modifications and been recommended for routine analysis, (10.5,10.6) This liquid reagent is based on the recommended method of the AACC. (10.7)

2.2 TEST PRINCIPLE



p-Nitrophenyl phosphate is hydrolyzed to p-nitrophenol and inorganic phosphate. The rate at which the p-Npp is hydrolyzed, measured at 405 nm, is directly proportioned to the alkaline phosphatase activity.

2.3 CLINICAL SIGNIFICANCE

Serum alkaline phosphatase estimations are of interest in the diagnosis of two groups of conditions: hepatobiliary disease, and bone disease associated with increased osteoblastic activity. (10.1)

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 Biotron's 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

(1X120,1X30 ml)

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

4.1 REAGENT

Alkaline Phosphatase working reagent contains:

magnesium acetate > 3.0 mM/L

p-nitrophenyl phosphate > 11.0 mM/L

Alkaline Phosphatase buffer contains

AMP buffer

4.1.1 Standard/Control/Calibrator > 0.3 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

The working reagent is prepared by mixing 4 parts of R1 to 1 part of R2.

4.4 REAGENT STORAGE AND STABILITY

Store reagent set at 2-8°C. Unopened reagents are stable until the expiration date is stored as directed. The working reagent is stable for 14 days at 2-8°C, and for 7 days at 18-26°C. Protect from direct light and avoid microbial contamination.

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 and pipettes.

4.5.4 Timer with one minute increments.

4.5.5 Constant temperature heat source which can be adjusted to 37° C.

4.5.6 Normal and abnormal control for quality control.

5.0 TEST PROCEDURE

The following is a general procedure for use on a manual instrument. Application procedures for use on an automated analyzers are available. Contact Biotron's Technical Service Department for specific information.

5.1 PROCEDURE CONDITIONS

Wavelength 405 nm
Temperature 37° C
Pathlength 1 cm
Mode Kinetic
Lag Time 1 min.
Sample to reagent ratio 1:40

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 1 ml of working reagent. Mix and incubate for 1 minute at 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 =
 $\Delta A / \text{min} \times \text{assay volume (ml)} \times 1000$

----- = $\Delta A / \text{min} \times 2187$

18.8 X light path (cm) X 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

2187 = factor derived from constants in the equation

Example:

Alkaline Phosphatase U/L =
0.019 X 1.025 X 1000

----- = 0.019 X 2187 = 42 U/L

18.8 X 1 X 0.025

0.019 = change in absorbance per minute

1.025 = 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

Note: To convert to SI units (nkat/L) multiply U/L by 16.67.

6.0 INTERPRETATION OF RESULTS

6.1 EXPECTED VALUES (10.4)

The range of expected values is:

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

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

Mean (U/L)	SD (U/L)	CV (%)
94	± 1.93	2.1
319	± 4.28	1.3

Between-Run

Mean (U/L)	SD (U/L)	CV (%)
95	± 1.26	1.3
315	± 3.26	1.2

9.2 CORRELATION

A correlation study was done comparing this method and a similar alkaline phosphatase method. The samples range between 35 and 375 U/L.

Number of Samples	Regression Equation y=Biotron, x=Comparative	Correlation Coefficient
95	y = 1.2 x + 4.5	0.981

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 0.0003 ΔA/min per unit of concentration (U/L).

10.0 REFERENCES

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