

ALKALINE PHOSPHATASE (IFCC-AMP)

INTENDED USE

Bioline Alkaline Phosphatase is used for the quantitative determination of Alkaline phosphatase in human serum.

CLINICAL SIGNIFICANCE

Distributed in almost every tissue of the body, serum alkaline phosphatase (ALP) levels are of interest in the diagnosis of hepato biliary disorder and bone disease. Most of the ALP in the normal adult serum is from the liver or biliary tract. Normal alkaline phosphatase levels are age-dependent, and are elevated during periods of active bone growth. Moderate elevations of ALP (not involving the liver or bone) may be attributed to Hodgkin's disease, congestive heart failure, and abdominal bacterial infections. Elevations also occur in the third trimester of pregnancy.

METHOD

Alkaline phosphatase is determined by measuring the rate of hydrolysis of various phosphate esters. p-Nitrophenyl Phosphate is one such ester that was used as a substrate by Fujita in 1939. Bowers and McComb further modified the procedure to a kinetic assay. In 1974, the Committee on enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology adopted a modification of the above procedure as the recommended procedure. The present method is a modification of the reference methods of the above committee and the American Association for Clinical Chemistry.

PRINCIPLE

The enzymatic sequence employed in the assay of Alkaline Phosphatase is as follows:

$$\text{p-Npp} + \text{H}_2\text{O} \xrightarrow{\text{Alk. Phos.}} \text{p-Nitrophenol} + \text{H}_3\text{PO}_4$$

p-Npp is colorless but p-Nitrophenol has strong absorbance at 405 nm. The rate of increased absorbance at 405nm is proportional to the enzyme activity.

REAGENT COMPOSITION

After combining R1 and R2 as directed, the reagent contains:

P-Nitrophenyl Phosphate	17 mM
Magnesium acetate	≤ 2 mM
Zinc sulfate	≤ 1 mM
HEDTA	≤ 2 mM
AMP Buffer	<350 mM

WARNINGS AND PRECAUTIONS.

- For *in vitro* diagnostic use.
CAUTION: In vitro diagnostic reagents may be hazardous. Handle in accordance with good laboratory procedures which dictate avoiding ingestion, and eye or skin contact.
- Specimens should be considered infectious and handled appropriately.

REAGENT PREPARATION.

The working reagent is prepared by mixing (4) volumes of R1 with (1) volume of R2 in a disposable container.

STORAGE AND STABILITY OF REAGENT.

Store reagent set at 2-8°C (refrigerated). The reagents are stable until the expiration date if stored as directed. Protect from direct light. Avoid microbial contamination.

REAGENT DETERIORATION

The reagent should be discarded if:

- Turbidity has occurred; turbidity may be a sign of contamination.
- The working reagent has an absorbance against water greater than 1.200 at 405 nm.

SPECIMEN COLLECTION AND STABILITY

Unhemolyzed serum is the preferred sample. Heparinized plasma may also be used. **Oxalate, fluoride and EDTA inhibit alkaline phosphatase, so are unsuitable as anticoagulants.** Samples should be kept cold and assayed as soon as possible after collection. A timed routine for sample collection and analysis should be established in each laboratory because ALP levels in serum or plasma, or in reconstituted control serum, rise significantly when stored at 2°-8°C or at room temperature.

Serum or heparin plasma Stability: 7 days at 2 – 8°C 2 months at –20°C Only freeze once. Discard contaminated specimens.

INTERFERING SUBSTANCES

EDTA, citrate, fluoride, and oxalate inhibit alkaline phosphatase. Young et al. gives a list of drugs and other substances, which may interfere with the determination of ALP activity.

ASSAY PROCEDURE FOR SEMIAUTO ANALYZER.

Wavelength 405nm

Temperature 37°C

	Test
ReagentR1	800µL
ReagentR2	200µL
Sample	25µL

Mix and aspirate the reagent into analyzer, after 60 sec of delay measure the optical density during next 90 sec. Calculate the $\Delta\text{Abs}/\text{min}$ for the sample.

CALCULATION

Alkaline Phosphatase activity IU/L = $\Delta\text{Abs}/\text{min} \times 2720$

Where: $\Delta\text{Abs}/\text{min}$ = Absorbance Change

LIMITATIONS

This methodology measures total alkaline phosphatase irrespective of tissue or organ of origin. Further tests may be necessary to assist in differential diagnosis.

QUALITY CONTROL

It is recommended that controls be included in each set of assays. Commercially available control material with established alkaline phosphatase values may be used for quality control. The assigned value of the control material must be confirmed, by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction, or procedural errors.

EXPECTED VALUES

Adults 40 - 129 IU/L at 37°C. Children have a higher normal value. It is strongly suggested that each laboratory establish its own normal range.

PERFORMANCE CHARACTERISTICS

- Linearity: 900 IU/L. For samples above linearity dilute the sample and multiply the result with dilution factor to get correct ALP value.
- Comparison: A group of 94 sera ranging in alkaline phosphatase values from 26.5 - 688 IU/L was assayed by this method and a similar commercially available reagent. Comparison of the results yielded a correlation coefficient of 0.997 and the regression equation was $y=1.290x-5.89$. (Comparison studies were performed according to NCCLS Tentative Guidelines, Ep9-T.)

3. Precision studies:

	Within Run	
<u>Mean IU/L</u>	<u>S.D.</u>	<u>C.V.(%)</u>
46.7	3.9	8.3
157.8	3.3	2.1

	Run-to-Run	
<u>Mean IU/L</u>	<u>S.D.</u>	<u>C.V.(%)</u>
44.4	2.8	6.4
157.0	3.1	1.9

General Technical Parameters

Mode	Kinetic
Wavelength (Filter)	405nm
Reaction Direction	Increasing
Sample Vol.	25µl
Reagent Vol.	1000 µl
Delay Time / Lag Time	60 Sec
Interval time	30 Sec
No of readings	3 Sec
Measuring Time	90 Sec
Reagent Blank Abs.(Max)	NMT 1.2
Calibration Method	Fix factor
Factor	2720
Linearity	900IU/L
Decimal Places	0
Temp.	37°C
Unit	IU/L
Ref. Low (Male / Female)	40 IU/L
Ref. High (Male / Female)	129 IU/L

REFERENCES

1. Kochmar, J.F., and Moss, D.W.: *Fundamentals of Clinical Chemistry*, N.W. Tietz (ed), p. 604, W.B. Saunders and Company, Philadelphia, PA (1976).
2. Bowers, G.N., Jr., McComb, R.B.: A Continuous Spectrophotometric Method for Measuring the Activity of Serum Alkaline Phosphatase. *Clin. Chem.* 12:70 (1966).
3. Tietz, N.W., (ed): Study Group on Alkaline Phosphatase. A Reference Method for measurement of alkaline phosphatase activity in Human Serum. *Clin. Chem.* 29:751 (1983).
4. Young, D.S., et al: *Clin. Chem.* 21:5 (1975).
5. Tietz, N.: *Fundamentals of Clinical Chemistry* 602-609 (1976).