

Alkaline Phosphatase Reagent

Catalog #: 43702

for use with the

SDI CA480 Clinical Chemistry System

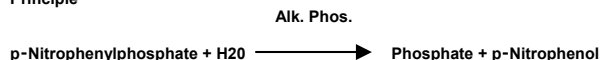
INTENDED USE For the in vitro quantitative determination of Alkaline Phosphatase in human serum.

SUMMARY AND EXPLANATION ^{1,2}

Alkaline phosphatase is a hydrolytic enzyme found in serum in numerous distinct forms which originate mainly from bone and liver. Physiological increases are found during bone growth in childhood and in pregnancy, while pathological increases are largely associated with hepatobiliary and bone diseases. Elevated activities are also observed in infectious hepatitis, bone disease, osteomalacia (rickets), bone metastases and hyperparathyroidism.

METHODOLOGY 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 1939.³ Bessey, Lon and Brock published an endpoint procedure in 1946⁴ while Bowers and McComb reported a kinetic procedure in 1966.⁵ The SDI method is based on the kinetic photometric test, according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

Principle



REAGENT COMPOSITION

Active Ingredients	Concentrations
Reagent 1	
2-Amino-2-methyl-1-propanol	0.35 mM
Magnesium Sulfate	2.0 mM
Zinc Sulphate	1.0 mM
HEDTA	2.0 mM
Reagent 2	
p-Nitrophenylphosphate	16.0 mM
pH 10.4 ± 0.1	

Concentrations are those in the working reagent.

Precautions and Warnings:

- For in vitro diagnostic use only.
- DO NOT pipette by mouth. Avoid contact with skin and eyes. If spilt, thoroughly wash affected area with water. For further information, consult the SDI Alkaline Phosphatase Reagent Material Safety Data Sheet.
- Reagent contains Sodium Azide as a preservative. In a dry state may react with copper or lead plumbing to form explosive metal azides. Upon disposal, flush with large amounts of water to prevent azide build up.
- Do not use the reagent after the expiration date printed on the kit.

REAGENT PREPARATION

Reagents are supplied in a two vial, ready to use, liquid form.

For some analyzers, the reagents can be combined to make a working solution by mixing 4 parts of Reagent 1 with 1 part of Reagent 2 (e.g., 20mL Rgt 1 to 5mL Rgt 2). For instructions on use, consult the specific JAS Instrument Application Sheet for your instrument.

REAGENT STORAGE

- Store the reagents at 2-8°C (refrigerated).
- The reagents are stable until the expiration date when stored at 2-8°C.
- Working reagent is stable for 4 weeks when stored at (2-8°C).
- Do not freeze the reagents.
- Reagent 2 should be protected from light.

REAGENT DETERIORATION

DO NOT USE THE REAGENT IF:

- The reagent is turbid.
- The reagent has an optical density greater than 1.0 at 405 nm.

SPECIMEN COLLECTION AND STORAGE

- Use non-hemolyzed serum or heparin plasma.
- Serum samples should be stored at 2-8°C and run within seven days.

INTERFERENCES

Studies to determine the level of interference for hemoglobin, bilirubin, lipemia, and ascorbic acid were carried out, the following results were obtained:

Hemoglobin:

No significant interference (± 10%) from hemoglobin up to 1000 mg/dL.

Bilirubin:

No significant interference (± 10%) from bilirubin up to 25.8 mg/dL.

Lipemia:

No significant interference (± 10%) from lipemia up to 842 mg/dL measured as triglycerides.

A number of drugs and substances may affect the accuracy of this test. See Young, et al.⁶

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- A SDI CA480 Clinical Chemistry System
- Deionized water and related equipment, e.g.: pipettes
- Analyzer specific consumables, e.g.: sample cups
- Control material such as those provided by SDI Biomed

ASSAY PROCEDURE

System Parameters

Alkaline Phosphatase (Liquid)

TEMPERATURE:	37°C
WAVELENGTH:	405 nm
ASSAY TYPE:	Rate/Kinetic
DIRECTION:	Increase
e.g. Sample Vol.	0.020 mL (20 mL)
Reagent 1 Vol.	1.0 mL (1000 mL)
Reagent 2 Vol.	0.250 mL (250 mL)
DELAY/LAG TIME:	1 Min
READ TIME:	3 Min

Procedure Notes:

- The reagent and sample volumes may be altered proportionally to accommodate various instrument requirements
- Samples with values above 1500 U/L should be diluted 1:1 with saline, re-assayed and the results multiplied by two
- If the spectrophotometer being used is equipped with a temperature controlled cuvette, the reaction mixture may be left in the cuvette while the absorbance readings are taken.

Calculations One Unit (U/L) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under the specified conditions.

$$I \text{ U/L} = \frac{A \text{ Abs./min.} \times 1000}{18.75 \times 1 \times 0.020} \times 1.270 = A \text{ Abs./min.} \times 3387$$

Where: AAbs /min.	= Absorbance change
1000	= Conversion of U/ml to U/L
1.270	= Total reaction volume (mL)
18.75	= Millimolar absorptivity of p-Nitrophenol
1	= Light path in cm
0.020	= Sample volume (mL)

Example: If your DAbs / min. = 0.06
then 0.06 x 3387 = 205 U/L.

NOTE:

If test parameters are altered the factor has to be recalculated using the above formula. To convert to SI units (nkat/L) multiply U/L by .01667.

CALIBRATION The procedure is standardized by means of the millimolar absorptivity of p-Nitrophenol (18.75 at 405nm) under the specified conditions. Results are based on the change in absorbance per unit of time, all parameters must be known and controlled.

QUALITY CONTROL The integrity of the reagent should be monitored by use of a two level control with known Alkaline Phosphatase values.

EXPECTED VALUES (37-C) ^{7,8}

Adults	
Women 20-50 years	42 - 98 U/L.
Men 20-50 years	53 - 128 U/L.
Women >60 years	53 - 141 U/L.
Men >60 years	56 - 119 U/L.

Children have a higher normal value.

It is strongly suggested that each laboratory establish its own normal range.

PERFORMANCE

Linearity:

When run as recommended the assay is linear from 2 to 1500 U/L.

Method Comparison:

Studies performed between this procedure and a similar procedure yielded the following results:

Number of samples pairs:	49
Data Range:	9 – 1501 U/L
Correlation Coefficient:	0.9992
Slope:	0.962
Intercept:	2.9 (U/L)

Precision:

Within Run	Level 1	Level 2	Level 3
n=40			
Mean (U/L)	30.6	66.7	197.8
S.D. (U/L)	0.07	0.8	1.7
C.V. (%)	2.2	1.2	0.9

Total

n=40			
Mean (U/L)	30.6	66.7	197.8
S.D. (U/L)	1.2 1.8	2.6	
C. V. (%)	4.1 2.7	1.3	

Sensitivity:

The sensitivity for this reagent when run as recommended is 0.24 DmA / min per U/L.

REFERENCES

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Manufactured for:



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