



## ALKALINE PHOSPHATASE-SL ASSAY

**CATALOGUE NUMBER:** 328-10  
328-30

**SIZE:** R1: 1 x 100 mL, R2: 1 x 25 mL  
R1: 3 x 100 mL, R2: 1 x 75 mL

### INTENDED USE

For the quantitative determination of alkaline phosphatase in serum. For IN VITRO diagnostic use.

### PRECAUTIONS

Avoid ingestion and contact with skin and eyes. See Material Safety Data Sheet.

### REAGENTS

R1: ALP-SL Buffer Reagent, R2: ALP-SL Substrate Reagent

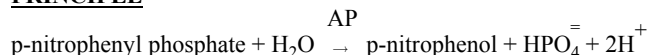
Concentrations in the test: 0.84 mol/L 2-amino-2-methyl-1-propanol (pH 10.4 at 25°C), 2.0 mmol/L magnesium acetate, 1.0 mmol/L zinc sulphate, 2.0 mmol/L HEDTA, 50 mmol/L p-nitrophenylphosphate, 50 mmol/L phenol and a preservative.

### HISTORY

Elevated alkaline phosphatase activity in serum is of interest in the diagnosis of several general disease conditions including hepatobiliary disease and bone disease associated with increased osteoblastic activity. Alkaline phosphatase activity in serum may be elevated due to obstructive jaundice, occlusion of the common bile or hepatic duct, and cirrhosis. (1)

Alkaline phosphatase (ALP) activity was first measured by Kay (2). Since that time many substrates such as glycerol phosphate and phenyl phosphate have been used. Bessey, Lowry, and Brock (3) introduced a more sensitive substrate p-nitrophenyl phosphate (p-NPP). Several recommendations have been made on the optimum conditions for the determination of ALP in serum. These suggestions have been put forth by the German Society for Clinical Chemistry (4) as well as by the committee on enzymes of the Scandinavian Society for Clinical Chemistry (5). This method is a modification of the recommendations of the International Federation of Clinical Chemistry (IFCC) for ALP measurement in serum (6).

### PRINCIPLE



Alkaline phosphatase hydrolyzes p-NPP to form the yellow chromogen p-nitrophenol according to the equation.

The rate of increase in absorbance of the reaction mixture at 415 nm, due to the formation of p-nitrophenol, is proportional to the alkaline phosphatase activity.

### REAGENT PREPARATION

The reagents are provided in a ready to use format. An alkaline phosphatase working reagent can be prepared by mixing 4 parts ALP-SL Buffer Reagent (R1) with 1 part ALP-SL Substrate Reagent (R2).

### REAGENT STABILITY AND STORAGE

The reagents included are stable until the expiry date stated on the labels at 2-8°C. The working reagent is stable for 28 days at 2-8°C or 7 days at 18-26°C.

All stability claims are based on real time studies in Diagnostic Chemicals Limited laboratories.

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## **QUALITY CONTROL**

A normal and abnormal level control serum should be analyzed with each run of samples and the results should fall within plus or minus two standard deviations of the established value. The best results are obtained if the alkaline phosphatase assay is performed at the same time interval following reconstitution of the control serum each day ( 9, 10).

## **CALCULATION AND RESULTS**

### **Results**

Alkaline phosphatase activity is expressed as units per liter (U/L).

### **Limitations**

A sample with an alkaline phosphatase level exceeding the linearity limit should be diluted with 0.9% saline and reassayed incorporating the dilution factor in the calculation of the value.

### **EXPECTED VALUES** (11)

Less than 103 U/L (30°C)

Less than 138 U/L (37°C)

These values are suggested guidelines. Alkaline phosphatase levels have been found to vary with age and sex. It is recommended that each laboratory establish the normal range for the area in which it is located.

## **PERFORMANCE CHARACTERISTICS**

These performance characteristics were generated in DCL laboratories using automated procedures unless otherwise stated.

### **Recovery Study**

Alkaline phosphatase was added to pooled human sera to increase the alkaline phosphatase concentration by 70 U/L, 89.5 U/L, and 148 U/L. Recovery of the added alkaline phosphatase averaged 95.7%.

### **Reportable Range** (NCCLS EP6-P)

Reportable range is dependent on the sample to reagent ratio used. A sample to reagent ratio of 1:70 gives a reportable range from 4.3 to 2000 U/L

### **Precision Studies** (NCCLS EP5-T2)

Data was collected on two control sera using a single lot of reagent in 40 runs conducted over 20 days.

Sample	Mean (U/L)	Total SD (U/L)	Total CV %	Within Run SD (U/L)	Within Run CV %
Serum 1	47	2.1	4.6	1.1	2.4
Serum 2	214	7.4	3.5	2.1	1.0

### **Accuracy** (NCCLS EP9-P)

The performance of this method (y) was compared with the performance of a similar alkaline phosphatase method (x) on a Hitachi 717 analyzer. Forty patient serum samples ranging from 16-1895 U/L gave a correlation coefficient of 1.0000. Linear regression analysis gave the following equation:

$$\text{This method} = 1.0276 (\text{reference method}) - 1.0 \text{ U/L.}$$

## **REFERENCES**

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