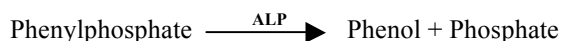# Alkaline Phosphatase

End Point Determination
Store at 2- 8ºC

PRINCIPLE

Colorimetric determination of the ALP activity according to the following reaction:

$$Phenylphosphate \xrightarrow{ALP} Phenol + Phosphate$$

The phenol liberated is measured in the presence of amin 4 antipyrine and potassium ferricyanide.
The presence of sodium arsenate in the reagent stops the enzymatic reaction

REFERENCE VALUES

Adults	21 – 92 U/l
Children	71 – 142 U/l

Children have a higher normal value. It is strongly suggested that each laboratory establish its own normal range.

SAMPLES

Serum or heparinized plasma can be used. Stable for 7 days at 2-8°C. The activity increases if samples are left at room temperature for several hours. Hemolysis will interfere.

REAGENTS

R1:

Disodium phenylphosphate	5.0	mmol/l
Carbonate-bicarbonate buffer	50	mmol/l

R2:

Phenol	142 U/l

R3:

Amino 4 antipyrine	60	mmol/l
Sodium Arsenate	240	mmol/l
TOXIC. CORROSIVE REAGENT Use an automatic pipette. Avoid contact with the skin		

R4:

Potassium Ferricyanide	150 mmol/l

PREPARATION OF WORKING REAGENT

All reagents are ready to use.

PROCEDURE

Wavelength	510 nm (Hg 492)
Zero adjustment	Reagent Blank

Set up the following tubes:

	Serum sample	Serum Blank	Standard	Reagent Blank
Reagent 1	2 ml	2 ml	2 ml	2 ml
Incubation for 5 minutes at 37 C				
Serum	50 µl	-	-	-
Reagent 2	-	-	50 µl	-
Incubation for exactly 15 minutes at 37 C				
Reagent 3	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Mix well				
Reagent 4	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Serum	-	50 µl	-	-
D-water	-	-	-	50 µl

Mix. Let stand for 10 minutes in the dark
Measure.
The color intensity is stable 45 minutes

CALCULATION

$$ALP\ U/l = \frac{(A)\ serum\ sample - (A)\ serum\ Blank}{(A)\ Standard} \times 142$$

LINEARITY

Any sample that gives a value >285 U/L should be diluted.

BIBLIOGRAPHY

Kind P.R.N, King E.G J.Clin. Path 1954-7-322
BELFIELD A, GOLDBERG D.M Enzyme 1971-12-561

The following symbols are used on labels

IVD	For in vitro diagnostic use
	Use day (last day of the month)
	Temperature limitation
LOT	Batch code
REF	Code