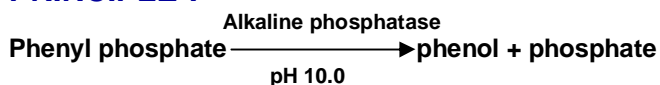


## ALKALINE PHOSPHATASE ( EC 3. 1. 3. 1 )

**Colorimetric Method**

**50 Tests**

### PRINCIPLE :



The liberated phenol is measured colorimetrically in the presence of 4- aminophenazone and potassium ferricyanide .

### SAMPLE :

Serum, Hemolysis will interfere .

### REAGENTS :

1.	<b>Standard phenol</b>	1.59 mmol / L
2.	<b>Buffer – Substrate :</b> Buffer pH 10.0 Phenyl phosphate	50 mmol / L 5 mmol / L
3.	<b>Enzyme Inhibitor :</b> EDTA 4-Aminophenazone	100 mmol / L 50 mmol / L
4.	<b>Color Reagent :</b> Potassium ferricyanide	200 mmol / L

### STABILITY :

The reagents are stable up to the expiry date specified when stored at +4 to +8 °C .

### PROCEDURE :

	Standard ( mL )	Sample ( mL )	Blank ( mL )
Reagent 1	0.025	-	-
Serum	-	0.025	-
Reagent 2	0.50	0.50	0.50
Incubate for 20 min. exactly at 37°C, then add:			
Reagent 3	0.25	0.25	0.25
Mix well, then add :			
Reagent 4	0.25	0.25	0.25

Mix , let stand for 5 min. at room temp. in the dark. Read the absorbances of sample ( $A_{\text{Sample}}$ ) and standard ( $A_{\text{standard}}$ ) against reagent blank at 510 nm. The color is stable for one hour . The reaction is linear up to 250 IU / L.

### CALCULATION :

$$\text{Enzyme activity ( IU / L )} = \frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times 75$$

### NORMAL VALUES :

Children : 70 – 140 IU / L .

Adults : 20 – 100 IU / L .

### REFERENCE :

Belfield A. and Goldberg D.M. (1971), Enzyme . 12,561.

### QUALITY CONTROL :

For accuracy and reproducibility control:- Assayed Multi – Sera, Normal and Elevated.

