

ALKALINE PHOSPHATASE

①

- It is a hydrolase - catalyses the hydrolysis of a chemical bond.

e.g.

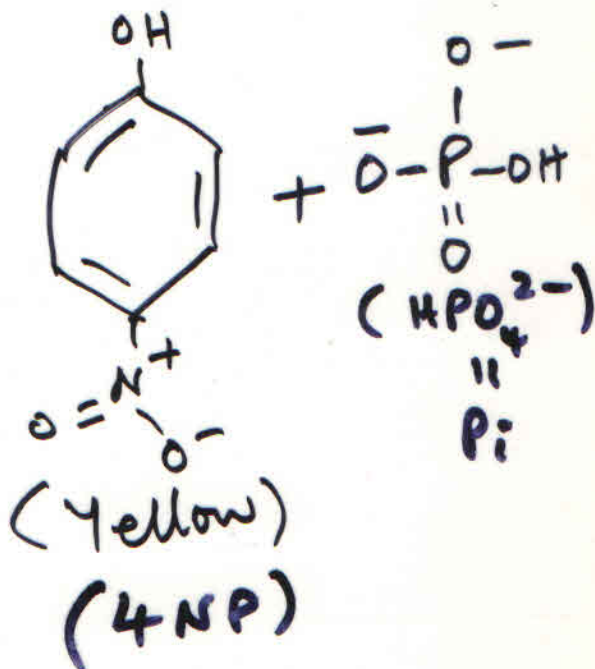
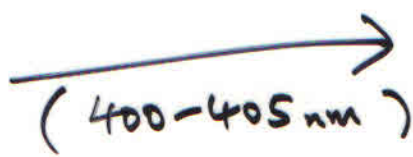
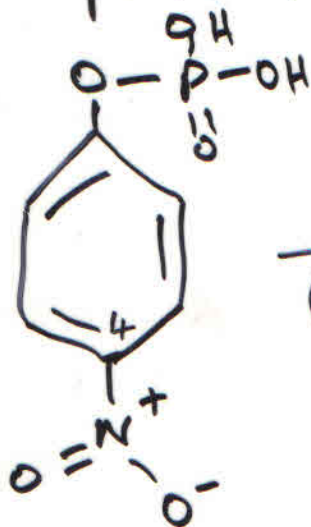


EC No. = 3 i.e. EC 3.1.3.1

- Removes phosphate groups from many types of molecules e.g. Nucleotides, Proteins \Rightarrow Dephosphorylation.

- Most effective ~~against~~ in an alkaline pH.

- ALP hydrolyses (4NPP), a chromogenic substrate to an organic radical (4NP) - which is yellow in color.



($C_6H_5NO_6P$)

(4-nitrophenyl) dihydrogen phosphate
(4NPP)

(Yellow)
(4NP)

- Occurs in 3 isoenzymes
 - ALP I = Intestinal
 - ALPL = Tissue Non-specific (Liver/Kidney/Bone)
 - ALPP = Placental
- Found in all tissues but more pronounced in liver, bile duct, kidney, bone and placenta.

- Normal high levels
 - Children
 - Pregnant women

- Abnormal high levels
 - Blocked bile ducts
 - Osteoblast activity in bone e.g. Paget's disease
 - Cholestasis (liver)
 - Cirrhosis (liver)
 - Hepatitis
 - Drug intoxication
 - Cholecystitis
 - Osteosarcoma
 - Vit D deficiency = Osteomalacia/Rickets
 - Prostate cancer arising from prostate to bone (metastases)
 - Bone fracture

Low levels

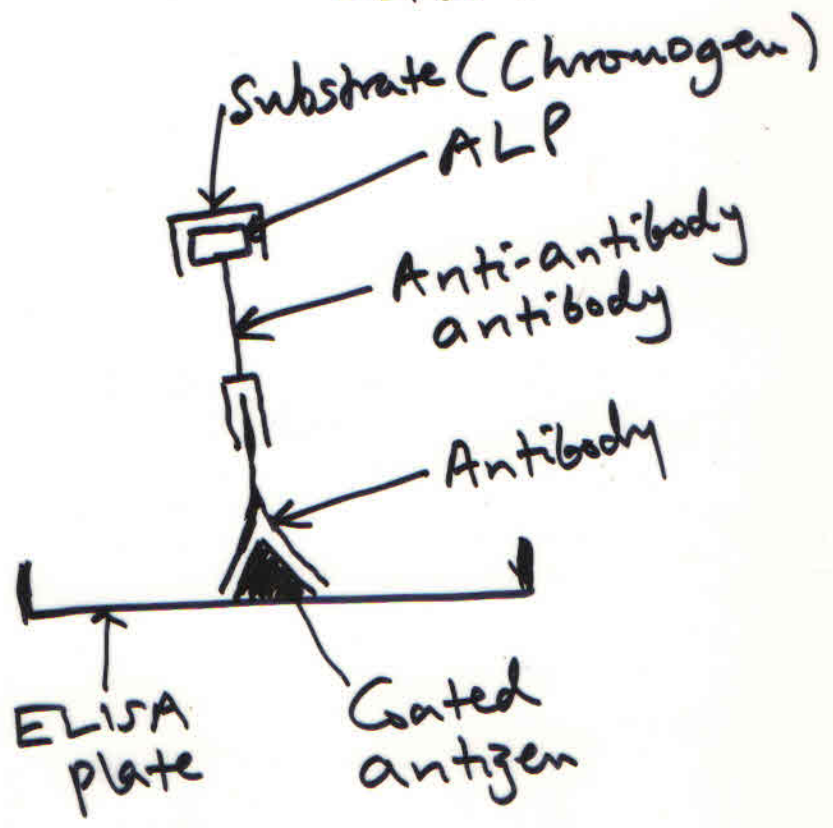
- Leukemias
- Pernicious anemia
- Hypophosphatasia
- Postmenopausal women receiving estrogen therapy
- Enteritis

Abnormal low levels

- Hypophosphatasia.
- Men with recent heart surgery.
- Postmenopausal women receiving estrogen therapy.
- Several types of anemia.

Commercial use

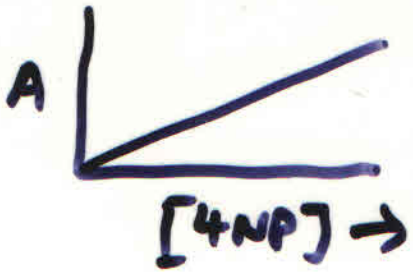
- ① Enzyme immunoassays e.g. ELISA
- ② Marker of pasteurisation = Aschaffenburg Mullen Test (AMT) on milk.
Raw milk → Turns yellow
Pasteurised milk → No change in color.



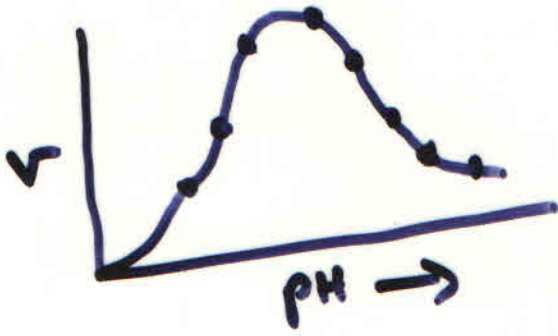
ALP ASSAY

①

① A: STD curve



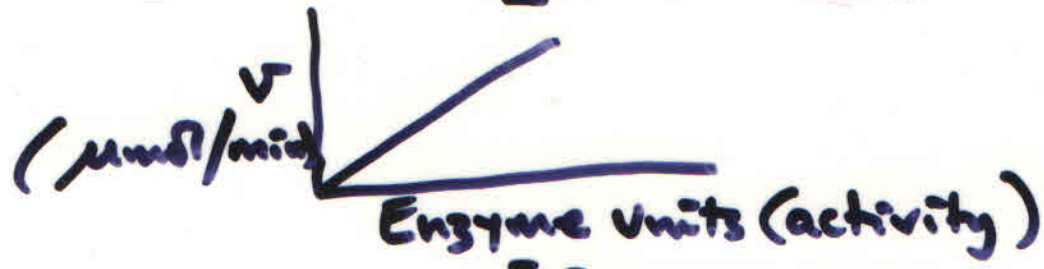
B: PH activity profile



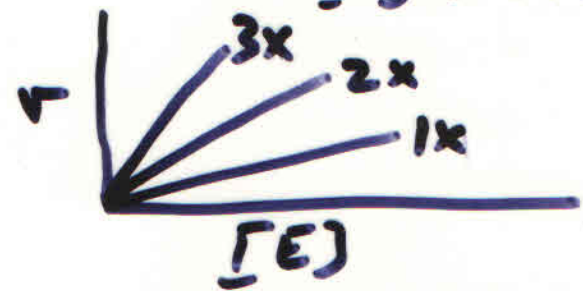
C: Time course



D: Effect of [E] i.e. [E] vs Rate (v)



[S] = $\times 10$ mM



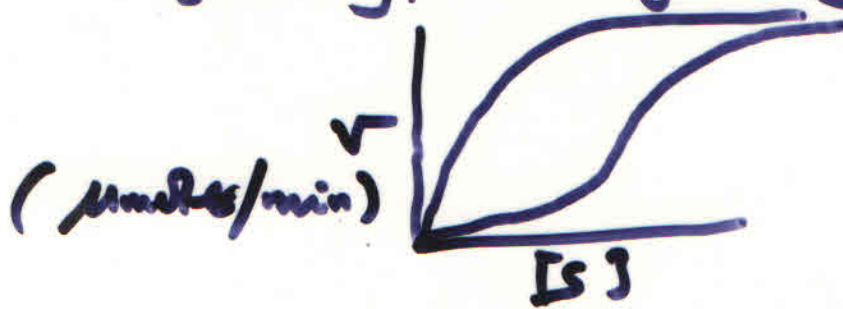
[E] = No. of active sites
 $\uparrow [E] = \uparrow [v]$ STEADY STATE

ALP

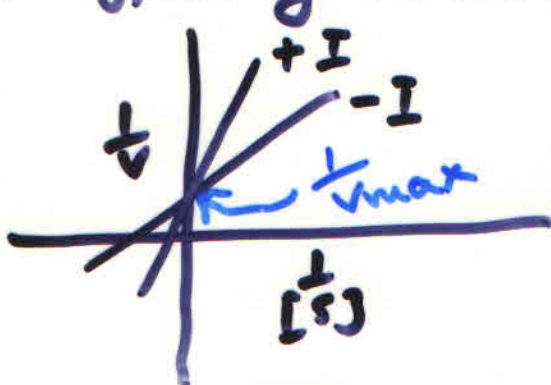
(2)

E: Effect of $[S]$ i.e. $\uparrow[S] = \uparrow[v]$

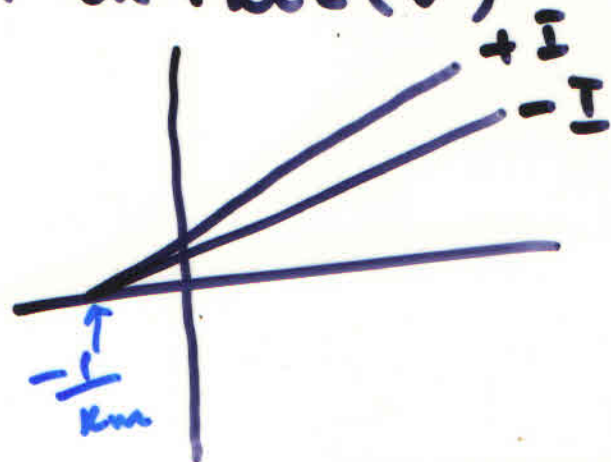
* Regulatory / Non-regulatory enzyme



F: Effect of Inhibitor on rate (v)



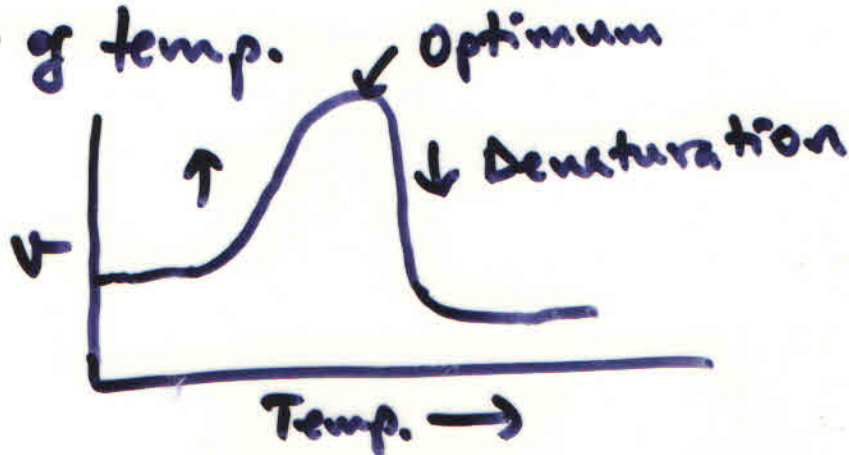
COMPETITIVE



NON-COMPETITIVE

$$\text{Slope} = \frac{K_m}{v_{\max}}$$

G: Effect of temp.



NB

Activity = $\mu\text{moles/min}$

Specific activity = $\mu\text{moles/min/mg protein}$