

Quantitative Demonstration of alkaline phosphatase (ALP)

CLS431

Principle of the method

+ p-Nitrophenylphosphate

- PH=10.4 ALP
- → p-Nitrophenol + phosphate + H₂O

The increase of absorbance is directly proportional to the amount of ALP present in the sample

Clinical Significance

- Highest activities are seen in liver, bone, intestine, placenta and kidney.
- Elevations of serum ALP are of diagnostic significance in the evaluation of hepatobilliary disease.
- Normal ALP levels are age dependent and are elevated during periods of active bone growth.

Clinical use

- ★ Causes of increased plasma ALP:
- * Paget's disease of bone
- + Osteomalacia
- ♦ Obstructive liver disease
- Hepatitis
- hepatotoxicity caused by drugs
- → Causes of decreased plasma ALP:
- + Cretinism
- + Vitamin C deficiency.

- ♦ Procedure
- * Assay conditions:
- → Wavelength 405nm.

- * Adjust the instrument to zero with distilled water or air.

* Pipette into a cuvette:

WR (ml)	1.0
Pre-warm at 37 C and add:	
Sample (µl)	20

- ♦ Mix, incubate for 1 minute at 37 C
- Read initial absorbance of the sample, start the the stopwach and read absorbances at 1 minute intervals thereafter for 3 minutes.
- ϕ Calculate the difference between absorbances and the average absorbance differences per minute ($\Delta A/min$)

+ Calculations

♦ ΔA/min X 2720= U/L of ALP

* Reference values:

* Men: 50 - 119 U/L

♦ Women: 43 - 110 U/L