



# Alkaline Phosphatase

DGKC optimized  
Kinetic method  
Stable liquid

## PRINCIPLE

Alkaline phosphatase catalyzes the hydrolysis of p-nitrophenyl phosphate, in the presence of magnesium ions, liberating inorganic phosphate and p-nitrophenol. The rate of p-nitrophenol formation is proportional to the concentration of ALP present in the sample.

## SAMPLE

Serum or Heparin plasma.  
Stable for at least 7 days at 2-8°C.

## REAGENT: Preparation of working reagent

R1.	DEA Buffer pH 10.2	1.25mmol/l
	Magnesium Chloride	0.625mmol/l
R2.	4-nitrophenyl phosphate	50mmol/l

## One reagent procedure

Mix 4 volumes of reagent 1 (R1) with 1 volume of reagent 2 (R2). Stability: 5 days at 20-25°C and 4 weeks at 2-8°C.

## Two reagents procedure

The reagents are ready for use.

## PROCEDURE

Using distilled water zero the instrument at 405nm (410).

## One reagent procedure

	Unknown
Sample	20µl
Working reagent	1.0 ml

Mix and after 1 minute incubation at 37°C (or 25-30°C). measure the change of absorbance per minute ( $\Delta A/\text{min}$ ) for a period of 3 minutes.

## Two reagents procedure

	Unknown
Sample	20µl
R1	1.0ml

Mix and wait 1 minute

R2. 250 µl

Mix and after a 1 minute incubation at 37°C (or 25-30°C), measure the change of absorbance per minute ( $\Delta A$ ) for a period of 3 minutes.

## Calculation

	One reagent	Two reagent
405 nm Activity (U/L) = $\Delta A/\text{min} \times$	2750	3 424
410 nm Activity (U/L) = $\Delta A/\text{min} \times$	2910	3 623

If results are to be expressed as SI units:  
 $\text{U/L} \times 16.67 = \text{nkatal/l}$

## LINENRITY

If  $\Delta A/\text{min}$  exceeds 0.250 at 402 nm, repeat test using serum diluted 1/10 with saline solution (9 g/L). multiply the result by 10.

## NORMAL VALUES

Serum 25°C  
Children: 110-720 U/L  
Adults: 60-170 U/L

Serum 30°C  
Children: 145-950 U/L  
Adults: 80-220 U/L

Serum 37°C  
Children: 180-1200 U/L  
Adult: 100-290 U/L

## NOTES

These reagents contain 0.1% sodium azide.  
Avoid contamination by using clean laboratory material (pipettes, plastic vials for analysers...)

## REFERENCES

- Scandinavian Society for Clinical Chemistry and Clinical Physiology. Recommended methods for the determination of four enzymes in blood. Scand. J. Clin. Lab. Invest. 1974; 33:291.

## ATLAS MEDICAL

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