

Quantitative determination of Alkaline Phosphatase in Serum/plasma
Only for *In Vitro* Diagnostic use

ORDER INFORMATION

REF	Cont.
ALP 125	5 X 25 ML
ALP 200	4 X 50 ML
ALP 25	1 X 25 ML

CLINICAL SIGNIFICANCE

Alkaline phosphatase is a hydrolytic enzyme found in serum in numerous distinct forms which originate mainly from bone and liver. Physiological increases are found during bone growth in childhood and in pregnancy, while pathological increases are largely associated with hepatobiliary and bone diseases. Elevated activities are also observed in infectious hepatitis, bone disease, osteomalacia (rickets), bone metastases and hyperparathyroidism.

PRINCIPLE

Alkaline phosphatase (ALP) catalyses the hydrolysis of p-nitrophenyl phosphate at alkaline pH, liberating p-nitrophenol and phosphate. The rate of p-Nitrophenol formation, measured photometrically, is proportional to the catalytic concentration of alkaline phosphatase present in the sample.

REAGENT COMPOSITION

Reagent I : Buffer reagent
Reagent II : Substrate reagent

SAMPLE COLLECTION AND PRESERVATION

Serum: Use non - haemolysed serum.

Plasma: Use heparin. Do not use EDTA, Oxalate or Fluoride.

Alkaline phosphatase in serum or plasma is stable for 7 days at 2-8°C

REAGENT PREPARATION

A single working reagent may be prepared by mixing four parts R1 Buffer Reagent with one part R2. Substrate Reagent.

REAGENT STORAGE AND STABILITY

The working reagent is stable for 7 days at 2-8°C.
Supplied reagent is stable at 2-8°C until expiry date.

AUTOMATED PARAMETERS	
Wavelength	405 nm
Cuvette	1 cm light path
Reaction Temperature	37°C
Measurement	Against distilled water
Reaction Type	Kinetic test
Reaction Direction	Increasing
Sample Volume	20 µl
Reagent Volume	1000 µl
Delay/Lag/time	60 Secs
Interval time	30 Secs
No. of Readings	04
Blank Absorbance limit	< 0.85
Factor	2720
Low Normal at 37°C	25 U/l
High Normal at 37°C	147 U/l
Linearity at 37°C	2000 U/l

ASSAY PROCEDURE

PIPETTE INTO TEST TUBES

Sample	20 µl
Working Reagent	1000 µl

Mix well and Incubate at 37°C for 60 secs. Measure absorbance increase every 30 secs for 2 minutes and determine the Δ A/min.

CALCULATION

$$A/min. \times 2720 = U/l \text{ Alkaline Phosphatase}$$

LINEARITY

The method is linear to a concentration of 2000 U/l. If the concentration exceeds this value, the sample should be diluted 1:1 with 0.9% saline solution and reassayed. Multiply the result by 2.

QUALITY CONTROL

It is recommended to run a normal and a pathological control serum which is commercially available to verify the performance of the measured procedure. The value of controls should fall within the established limit.

REFERENCE VALUES

ADULTS	25-147 U/l
*Children	
Aged 1 day	< 250 U/L
Aged 2-5 day	< 231 U/L
Aged 6 day – 6 Months	< 449 U/L
Aged 7Months-1 Year	< 462 U/L
Aged 1-3 Year	< 281 U/L
Aged 4-6 Year	< 269 U/L
Aged 7-12 Year	< 300 U/L
Aged 13-17 Year	< 390 U/L

* Calculated from published reference ranges for ALP opt. method (DGKC) using a factor of 0.417 derived from method comparison. The reference values are to be considered as indicative only. Every Laboratory should establish its own normal ranges.

BIBLIOGRAPHY

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