

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

ORDER INFORMATION

REF	Pack Size
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.

METHOD

Kinetic photometric test, according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

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

Reagent I : Buffer reagent
Reagent II : Substrate reagent

MATERIALS REQUIRED BUT NOT PROVIDED

- NaCl solution 9 g/L
- General laboratory equipment

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

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

WARNING AND PRECAUTIONS

- For in vitro diagnostic use.
- Do not use components beyond the expiration date.
- Do not mix materials from different kit lot numbers.
- Exercise the normal precautions required for handling all laboratory reagents.
- The reagent contains preservative. Do not swallow. Avoid contact with skin and mucous membranes.
- For detailed information refer Material Safety Data Sheet.

WASTE MANAGEMENT

Please refer to local legal requirements.

MATERIALS REQUIRED BUT NOT PROVIDED

- NaCl solution 9 g/L
- General laboratory equipment

SAMPLE COLLECTION AND PRESERVATION

Serum: Use non - haemolysed serum.

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

Do not use hemolytic samples!

Stability:

7 days at 4 – 8 °C

2 months at –20 °C in case of immediate freezing.

Freeze only once! Discard contaminated specimens!

ASSAY PROCEDURE

Operating Instructions

- Check reagent inventories at least daily to ensure that quantities are sufficient for the planned work load.
- Bring all reagents, standard and samples to room temperature 18 - 28°C, prior to analysis.

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 IU/L
High Normal at 37°C	147 IU/L
Linearity at 37°C	2000 IU/L

MANUAL ASSAY PROCEDURE

Pipette into Test Tubes

	Serum/Plasma	Urine
Sample	20 µl	10 µl
Reagent	1000 µl	1000 µl

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

SAMPLE DILUTIONS

- The method is linear to a concentration of 2000 IU/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.

CALCULATION

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

CALBRATORS AND CONTROLS

For the calibration of automated photometric systems the commercially available suitable multi-calibrator is recommended.

This method is traceable to the molar extinction coefficient.

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.

PERFORMANCE CHARACTERISTICS

WITHIN RUN

Sample	Mean Concentration	SD	CV %
Level 1	72.29	2.59	3.58%
Level 2	204.03	4.17	2.04%

RUN TO RUN

Sample	Mean Concentration	SD	CV %
Level 1	72.51	2.19	3.02%
Level 2	202.37	3.61	1.78%

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.

Limit of detection: The limit of detection for Alkaline Phosphatase is 2U/L.

METHOD COMPARISON

A comparison of Accucare Alkaline Phosphatase with a commercially available assay (x) using 59 samples gave following results: $R^2 = 0.9900$

REFERENCE VALUES

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

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

LIMITATION OF THE PROCEDURE

- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.











INTERFERENCE


- Bilirubin: No interference found upto Bilirubin 40mg/dl.
- Hemoglobin: No interference found upto Hemoglobin 400mg/dl.
- Lipemia: No interference found upto 900mg/dl.
- These characteristics have been obtained using an automatic analyzer. Results may vary if a different instrument or a manual procedure is used.

BIBLIOGRAPHY

- Zilva JF, Pannall PR, "Plasma Enzymes in Diagnosis" in Clinical Chemistry in Diagnosis and Treatment. Lloyd London 1979:Chap 15 343.
- IFCC method for the measurement of ALP J Clin Chem Clin Biochem 1983: 21:731-48.
- Young DS. Effects of Drugs on Clinical Laboratory Tests. Third Edition 1990: 3: 19-25.
- Tietz Textbook of Clinical Chemistry and Molecular Diagnosis (4th Ed.) Burtis, Ashwood & Bruns (Eds), Elsevier Saunders, 2005; 2290.

GLOSSARY OF SYMBOL

	Consult Instruction for Use		Lot Number
	Catalog Number		Date of Manufacturing
	Store between		Use By or Expiration Date
	Manufacturer		For <i>in vitro</i> Diagnostic use only
	Keep away from sunlight		Content of the kit

 LAB-CARE DIAGNOSTICS (INDIA) PVT. LTD.
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