ALKALINE PHOSPHATASE (ALP) (9+1)

Diagnostic reagent for the in-vitro quantitative determination of ALP in human serum and plasma on both automated and manual system.

REF: V/AP05.010	50 tests	REF: V/AP04.025	100 tests
REF: V/AP02.025	50 tests	REF: V/AP04.050	200 tests
REF: V/AP02.050	100 tests	REF: V/AP08.050	400 tests
REF: V/AP05.020	100 tests	REF: V/AP05.100	500 tests

CLINICAL SIGNIFICANCE

Alkaline phosphatase (ALP) catalyzes the hydrolysis of a wide variety of physiologic and non-physiologic phosphoric acid esters in alkaline medium (pH optimum 10). The liver and biliary tract are the source of alkaline phosphatase in normal sera. Normal alkaline phosphatase levels are age dependent being higher in children and adolescents in comparison to adults. ALP is one of the tests of choice for evaluating cholestasis and obstructive juandice. Elevated levels are found in many diseases including hepatitis, cirrhosis, malignancy, and in bone diseases.

METHOD PRINCIPLE

Kinetic method according to the International Federation of Clinical Chemistry.
Alkaline phosphatase (ALP) hydrolyzes p-Nitrophenylphosphate (p-NPP) to p-Nitrophenol and phosphate.

$$\text{p-Nitrophenylphosphate} + H_2O \xrightarrow{\text{ALP}} \text{p-Nitrophenol} + \text{Phosphate}$$

The increase of absorbance per minute at 405 nm is proportional to the enzyme activity.

REAGENT COMPOSITION

Reagents	Composition
R1: Buffer	
- 2-Amino-2-Methyl-1-Propanol (pH 10.3)	- 2.0 mol/L
- $MgCl_2$	- 2.0 mol/L
R2: Substrate	
- p-Nitrophenylphosphate	- 16 mmol/L

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,..).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the **Lab.Vie** ALP reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

Lab.Vie.ALP reagent working solution is prepared as by mixing 9 volumes of buffer (R1) and 1 volume of substrate (R2), e.g. 900 µl R1 + 100 µl R2. After reconstitution working reagent is stable for 4 weeks at 2 – 8 ºC or 5 days at 15 - 25 ºC. All reagents are stable until expiration date stated on label when properly stored in an upright position and refrigerated at 2-8°C (do not freeze).

Deterioration
Lab.Vie.The ALP reagent considered damaged if it is turbid, Do not use ALP reagent if it is turbid or if the absorbance of the working reagent is more than 2.2 at 405 nm. Failure to recover control values within the assigned range may be an indication of reagent deterioration.

SPECIMEN COLLECTION AND PRESERVATION

Serum and Plasma
Nonhaemolyzed fresh serum is the preferred specimen. Heparin is the only acceptable anticoagulant. Complexing anticoagulants such as citrate, oxalate, and EDTA must be avoided.
Alkaline phosphatase activity may slowly increase in serum samples stored at room temperature. Previously frozen or lypholized sera may show a marked decrease in values immediately upon thawing or reconstitution. The activity then increases to the initial values, and the rate of this increase is time and temperature dependent.

Stability: 2 months at -20 ˚C ; 4 weeks at 4 – 8 ˚C;
7 days at 20 – 25 ˚C

SYSTEM PARAMETERS

Wavelength	405 nm (400 – 420 nm)
Optical path	1 cm
Assay type	Kinetic
Direction	Increase
Sample Reagent Ratio e.g: Reagent volume Sample volume	1:100 1 ml 10 µl
Temperature	37 ˚C or 30 ˚C
Equilibration time	1 min.
Zero adjustment	Against Air
Reagent Blank Limits	Low 0.2 AU High 2.2 AU
Sensitivity	5 U/L
Linearity	750 U/L

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes and automatic pipet
- Centrifuge and spectrophotometer

ASSAY PROCEDURE

	Assay
Working solution	1.0 ml
Specimen	10 µl

Mix, read initial absorbance after 1 minute, and start timer simultaneously. Read again after 1, 2 and 3 minutes. Determine the mean absorbance change per minute (ΔA/min).

CALCULATION

ALP (U/L) = ΔA/min x 5454

QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control serum of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact **Lab.Vie**. technical support.

PERFORMANCE CHARACTERISTICS

Precision	Within run (Repeatability)		Run to run (Reproducibility)	
	Normal level	High level	Normal level	High level
n	20	20	20	20
Mean U/L	177.7	359.7	178.5	365.5
SD.	1.71	1.5	1.82	1.86
CV. %	0.96	0.43	1.15	0.55

The results of the performance characteristics depend on the analyzer used.

Accuracy (Methods Comparison)
Result obtained from **Lab.Vie**. ALP reagent compared with commercial reagent of the same methodology performed on 20 human sera give a correlation of 0.988.

Sensitivity
When run as recommended, the minimum detection limit of the assay is 5.0 U/L.

Linearity
The reaction is linear up to ALP concentration of 750U/L. specimens showing higher concentration should be diluted 1+5 with physiological saline and repeat the assay (result × 6).

INTERFERING SUBSTANCES

Haemolysis

A 200 mg/dl haemoglobin results in a 10% negative bias.

Icterus
No significant interference up to bilirubin level of 40 mg/dL.

Lipemia
No significant interference from lipemia up to 1000 mg/dL.

EXPECTED VALUES

Serum/plasma	30°C	37°C
Males (20 – 50) YEARS	30-90 U/l	53-128 U/l
Males (≥ 60) YEARS	30-90 U/l	56-119 U/l
Females (20 – 50) YEARS	20-80 U/l	42-98 U/l
Females (≥ 60) YEARS	40-111 U/l	53-141 U/l
Children (1 – 12) YEARS	< 350 U/l	< 460 U/l

Temperature conversion factor is 1.22 (25 to 30 °C)
And 1.52 (25 to 37 °C).

DYNAMIC RANGE

5 - 750 U/L.

REFERENCES

1. Moss DW. Alkaline phosphates iso enzymes. Clin Chem. 1982;28:2007-2016 .
2. Moss DW, Henderson AR, Kachmar JF. Enzymes in:Tietz NW, ed. Fundamentals of clinical chemistry. 3 rd ed. Philadelphia: WB Saunders; 1987:346-421.
3. Tietz NW, Rinker AD, Shaw LM. IFCC methods for the measurement of catalytic concentration of enzymes. Part 5. IFCC method for alkaline phosphatase . J Clin Chem Clin Biochem. 1983;21:731-748. 4.Zawta B, Klein G, Bablok W. Temperaturumrechnung in der Klinischen Enzymologie? Klin lab. 1994:40:23-32. Sensitivity

SYMBOLS IN PRODUCT LABELLING

IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		