Alkaline Phosphatase

(4+ 1)

INTENDED USE

Vitro ALP reagent is intended for the in vitro quantitative determination of alkaline phosphatase in serum and plasma on both automated and manual systems.

METHOD

Kinetic method according to the recommended reference method of DGKC.[1] Liquid stable double reagent.

CLINICAL SIGNIFICANCE

Alkaline phosphatase refers to a group of phosphatases (pH optimum approximately 10) found in almost every tissue in the body. Most alkaline phosphatase in normal adult serum is from the liver or biliary tract. Normal alkaline phosphatase levels are age dependent with young children and adolescents having much higher levels than adults. Adult males tend to have higher levels than females, but pregnant females have increased levels due to placental secretion of alkaline phosphatase.[2] Alkaline phosphatase in serum consists of four structural genotypes: the liver-bone-kidney type, the intestinal type, the placental type and the variant from germ cells. It occurs in osteoblasts, hepatocytes the kidneys, spleen, placenta, prostate, leukocytes and the small intestine. The liver-bone-kidney type is particularly important.[2] Elevation of alkaline phosphatase levels occurs in diseases such as hepatitis, cirrhosis, malignancy, chemical toxicity, and in bone diseases such as metastatic carcinoma, rickets, Paget's disease, and osteomalacia.[3] Moderate increase in serum alkaline phosphatase levels have been observed in Hodgkin's disease, congestive heart failure, ulcerative colitis, regional enteritis, and intra-abdominal bacterial infections. Alkaline phosphatase levels are normally elevated during periods of active bone growth, for example, in young children and adolescents[3].

ASSAY PRINCIPLE

Alkaline phosphatases catalyze the hydrolysis of a wide variety of physiologic and non-physiologic phosphoric acid esters in alkaline medium. The natural substrates for these enzymes have not yet been identified. Thus, a variety of synthetic substrates have been used in assay methods for ALP, the selection of which has been largely a matter of convenience. Kay demonstrated the presence of ALP in blood using β-glycerophosphate as the substrate.[5] This method requires measuring the rate of phosphate liberation against the background level of endogenous phosphate. Phenyl phosphate was used as a substrate by King and Armstrong, in this method, the liberated phenol has been measured in a variety of ways including the use of Folin-Ciocalteau reagent[6], and 4-aminoantipyrine.[7]Alkaline phosphatase is determined by measuring the rate of hydrolysis of various phosphate esters. p-Nitrophenyl phosphate is one such ester that was used as a substrate by Fujita in 1939.[7] Bowers and McComb further modified the procedure to a kinetic assay.[1] In 1967, Hausamen et al improved the method using diethanolamine as buffer.[10]

The vitro ALP reagent is based on the recommendation of the DGKC[1]. The series of reactions involved in the assay system is as follows:

Alkaline phosphatase (ALP) hydrolyzes the colorless p-nitrophenyl phosphate to p-nitrophenol and phosphate in the presence of magnesium ions. The product of enzyme hydrolysis p-nitrophenol, has a yellow color at the pH of the reaction.

$$\textbf{p-Nitrophenyl phosphate} + H_2O \xleftrightarrow[Mg^{2+}]{ALP} \textbf{p-Nitrophenol} + \textbf{Phosphate}$$

The rate of p-nitrophenol formation is directly proportional to the catalytic ALP activity. It is determined by measuring the increase in absorbance at 405 nm.

EXPECTED VALUES

25^{0}C	Male	Up to 180 U/l Up to 3.0 µkat/l
	Female	Up to 160 U/l Up to 2.67µkat/l
	Children Up12 years	Up to 720 U/l Up to 12.0 µkat/l
30^{0}C	Male	Up to 220 U/l Up to 3.67 µkat/l
	Female	Up to 195 U/l Up to 3.25µkat/l
	Children Up12 years	Up to 950 U/l Up to 15.84 µkat/l
37^{0}C	Male	Up to 270U/l Up to 4.5 µkat/l
	Female	Up to 240 U/l Up to 4.0µkat/l
	Children Up12 years	Up to 1200 U/l Up to 20.0 µkat/l

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range. For diagnostic purposes, the ALP results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

REAGENTS

R$_1$	Diethanolamine buffer pH 9.8	1.0 mol/l
	Magnesium ions	0.6 mmol/l
R$_2$	p-Nitrophenyl phosphate	10 mmol/l

- **Reagent Preparation & Stability**

All reagents are ready for use and stable up to the expiry date given on label when stored at 2–8^{0}C.

Prepare the working solution by adding one volume (1 ml) of **R$_2$** to four volumes (4 ml) of **R$_1$**. Mix well, do not shake, protect from direct light.

The working solution is stable for 4 months at 2–8 ^{0}C. Avoid microbial contamination

Serum, or heparinized plasma

SPECIMEN

- **Specimen Preparation & Stability**

Freshly collected serum specimen should be kept at room temperature and assayed as soon as possible but not later than 4 hours after collection. Freezing causes a loss of activity, which is slowly recovered at room temperature 18 to 24 hours after the sera are thawed.

Complexing anticoagulants such as citrate, oxalate, and EDTA must be avoided. Alkaline phosphatase levels in serum, plasma rise significantly when stored at 2-8^{0}C or room temperature.

PROCEDURE

- **Manual Procedure**

Wavelength	405 nm
Cuvette	1 cm light path
Temperature	25, 30 or 37 ^{0}C
Zero adjustment	against air
Specimen	Serum or plasma

Pipette into test tube or cuvette	
Working solution	1000 µl
Serum or plasma	20 µl

Mix, incubate for 30 seconds, and start stopwatch simultaneously. Read again after exactly 1, 2, and 3 minutes.

- **Automated Procedure**

User defined parameters for different auto analyzers are available upon request.

CALCULATION

Determine the change in absorbance per minute (ΔA/min) from the linear portion of the reaction curve and calculate the ALP activity by using the following formulae:

U/l = 2757 × ΔA 405 nm/min

One international unit **(U)** is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under specified conditions.

The general formula for converting ΔA/min into U/l is:

$$U/l = \frac{\Delta A/min \times TV \times 1000}{*\sum \times SV \times LP}$$

Where:

TV Total reaction volume in ml

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SV Sample volume in ml
*∑ millimolar absorptivity of p-nitrophenol.
LP Cuvette path length in cm
1000 Conversion of U/ml to U/l

* millimolar absorptivity of p-nitrophenol at 405 nm= 18.75

- **Unit conversion**
  U/l x 16.67 x 10^{-3} = µkat/l
- **Temperature correction**
  Multiply the result by 1.22 if the assay performed at 25^{0}C but is to be reported at 30^{0}C.
  Multiply the result by 1.5 if the assay performed at 25^{0}C but is to be reported at 37^{0}C.
  Multiply the result by 1.23 if the assay performed at 30^{0}C but is to be reported at 37^{0}C.

QUALITY CONTROL

It is recommended that controls (normal and abnormal) be included in:

- Each set of assays, or
- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Commercially available control material with established ALP/AP values may be routinely used for quality control.
Failure to obtain the proper range of values in the assay of control material may indicate:

- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

The following corrective actions are recommended in such situations:

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact Vitro Technical Services.

INTERFERING SUBSTANCES

- **Anticoagulants:**
  Complexing anticoagulants such as citrate, oxalate, and EDTA must be avoided. The only acceptable anticoagulant is heparin.
- **Bilirubin:**
  No significant interference from free or conjugated bilirubin up to a level of 60 mg/dl.
- **Drugs:**
  Young[13] in 1990 has published a comprehensive list of drugs and substances which may interfere with this assay.
- **Haemolysis:**
  Haemoglobin levels higher than 250 mg/dl decrease the apparent ALP activity significantly.
- **Lipemia:**
  No significant interference.
- **Others:**
  Pathological high levels of albumin (7.0 g/dl) increase the apparent ALP activity significantly.

WARNING & PRECAUTION

- Vitro ALP reagent is for in vitro diagnostic use only. Normal precautions exercised in handling laboratory reagents should be followed.
- Warm up working solution to the corresponding temperature before use.
- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- Valid results depend on an accurately calibrated instrument, timing, and temperature control.

PERFORMANCE CHARACTERISTICS

Imprecision

Reproducibility was determined using in an internal protocol. The following results were obtained.

	Within Run		Between Day	
Control	Level I	Level I	Level II	Level II
Number of samples	40	40	40	40
Mean (U/l)	175	176	410	393
SD (U/l)	2.28	4.6	10.7	5.43
CV (%)	1.5	2.64	2.53	1.46

Method Comparison

Comparison studies were carried out using another similar commercially available method. Serum and plasma (Heparin) samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained:
Number of samples 45
Range of sample results 48 – 225 U/l
Mean of reference method results 100 U/l
Mean of ALP results 105 U/l
Slope 1.03
Intercept -0.33 (-0.006 µkat/L)
Correlation coefficient 0.9995

Sensitivity

The sensitivity is defined as the lower detection limit represents the lowest measurable ALP activity that can be distinguished from zero.

When run as recommended the sensitivity of this assay is 5 U/l or 0.08 µkat/l).

LINEARITY

When run as recommended, the assay is linear up to 900 U/l or 15 µkat/l.

If result exceeds 900 U/l or 15 µkat/l, specimen should be diluted 1+5 with 0.9% NaCl solution and reassayed. Multiply the result by 6.

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6. **The committee on Enzymes of The Scandinavian Society for Clinical Chemistry and Clinical Physiology (1974):** Recommended Methods for the determination of four enzymes in blood. Scand J Clin Lab Invest. 32: 29.
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SYMBOL DECLARATION

	Manufacturer
	Consult instructions for use
LOT	Batch code (Lot #)
REF	Catalog number
	Temperature limitation
IVD	In vitro diagnostic medical device
	Use by
	Caution. Consult instructions
	Keep away from light

ORDERING INFORMATION

REF	SIZE	REF	SIZE
10501	5 X 10 ml	10505	5 X 20 ml
10502	1 X 50 ml	10506	5 X 25 ml
10503	6 X 10 ml	10507	3 X 50 ml
10504	10 X 10 ml	10508	4 X 50 ml

Article # :105 -EN
Date of Revision : 03/2021

ISO 13485:2016 CE

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