ALP - L

DGKG-DEA kinetic method for the quantitative determination of alkaline phosphatase (ALP) in serum and plasma

CE

IVD

ORDER INFORMATION

REF	Kit size
GA4933 00	5x40 + 1x50 ml
KL4933 00	8x16 + 8x4 ml
BK4933 00	2x(40+10 ml)

INDICATION

The alkaline phosphatase is present in practically all tissues of the body, and it occurs at particularly high levels in intestinal epithelium, kidney tubules, bone, liver, and placenta. Although the precise metabolic function of the enzyme is not yet understood, it appears that the enzyme is associated with lipid transport in the intestine and with the calcification process in bone. The form present in the sera of normal adults probably originates mainly in the liver, with up to half the total activity coming from the skeleton. The respective contributions of these two forms to the total activity are markedly age dependent. Serum ALP measurements are of particular interest in the investigation of two groups of conditions: hepatobiliary disease and bone disease associated with increased osteoblastic activity. For many years, it was believed that ALP reaching the liver from other tissues (especially bone) was excreted into the bile and that the elevated serum enzyme activity found in hepatobiliary disease was a result of a failure to excrete the enzyme through the bile. However, it is now known that the response of the liver to any form of biliary tree obstruction is to synthetize more ALP. Intrahepatic obstruction of the bile flow also raises serum ALP, but usually to a less extent. Liver diseases that principally affect parenchymal cells, such as infectious hepatitis, typically also show only moderately elevated or even normal serum ALP levels. Among the bone diseases, the highest levels of serum ALP activity are encountered in Paget's disease. Only moderate rises are observed in osteomalacia, the levels slowly declining in response to vitamin D therapy. Primary hyperparathyroidism and secondary hyperparathyroidism are associated with slight to moderate elevations of ALP activity in serum. Very high enzyme levels are present in patients with osteogenic bone cancer. Transient elevations may be found during healing of bone fractures. Physiological bone growth elevates ALP in serum, and this accounts for the fact that in the sera of growing children one finds enzyme activity some 1.5 to 2.5 times that in normal adult serum. An increase of up to two to three times normal may be observed in women in the third trimester of pregnancy, although the interval is very wide and levels may not exceed the upper limit of the reference interval in some cases. The additional enzyme is of placental origin.

METHOD PRINCIPLE

Alkaline phosphatise (ALP) catalyze the hydrolysis of 4-nitrophenylphosphate (4-NPP) with the formation of free 4-nitrophenol and inorganic phosphate, acting the alkaline buffers as a phosphate-group acceptor.
The reaction is monitored kinetically at 405 nm by the rate of formation of 4-nitrophenol, proportional to the activity of ALP present in the sample.

$$\text{4-Nitrophenylphosphate} + Mg^{++} + H_2O \xrightarrow{\text{ALP}} \text{4-Nitrophenol} + P_i$$

The method follows the proposed optimised formulation of the DGKC.

COMPOSITION

REAGENT A:

DEA buffer pH 10.2	1.25 mol/l
Magnesium chloride	0.6 mmol/l
Preservatives	

REAGENT B:

4-nitrophenylphosphate	50 mmol/l
Preservatives	

PREPARATION OF REAGENTS

Bireagent procedure:
The reagents are liquids ready to use.

Monoreagent procedure:
Mix 4 parts of Reagent A and 1 part of Reagent B to obtain the working reagent (ex. 20 ml of RA + 5 ml of RB).

Storage and stability
Store at 2-8 °C. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label, if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

Working reagent is stable for 5 days at 20-25 °C or 30 days at 2-8 °C if protected from light.

ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l

SAMPLES

Serum or heparinized samples, free of hemolysis. Avoid use of anticoagulants like oxalate, EDTA and citrate as they inhibit enzyme activity by complexing Mg^{++}. Do not utilize hemolyzed sample.
Alkaline phosphatise in serum or plasma is stable for 7 days at 2-8 °C.

Specimen collection / Preanalytical factors
It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL

It is recommended to use commercial Quality Control sera with known ALP activity. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE

Working temperature	37 °C
Wavelength	405 nm (400-410 nm)
Optical path	1 cm
Reaction	kinetic (increase)

Allow the reagents to reach working temperature before using.

Bireagent procedure

Pipette into disposable or well clean cuvettes:

	Sample
Reagent A	800 µl
Sample	20 µl
Mix and incubate at 37 °C for 5 minutes, then add:	
Reagent B	200 µl
Mix and incubate at 37 °C. After 1 minute read the absorbance (A) at 405 (400-410) nm against water. Read absorbance again 1, 2, 3 minutes thereafter. Calculate ΔA/min.	

Monoreagent procedure

Pipette into disposable or well clean cuvettes:

	Sample
Working reagent	1000 µl
Incubate at 37 °C for 5 minutes, then add:	
Sample	20 µl
Mix and incubate at 37 °C. After 1 minute read the absorbance (A) at 405 (400-410) nm against water. Read absorbance again 1, 2, 3 minutes thereafter. Calculate ΔA/min.	

CALCULATION OF RESULTS

Activity (U/l) = ΔA/min x 2764
Activity (µkat/l) = U/l x 0.01667

REFERENCE VALUES

Children: up to 800 U/l (13.3 µkat/l)
Adults: up to 270 U/l (4.5 µkat/l)

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES

Precision
Within-run coefficients of variation have been calculated on replicates of two samples at different enzymatic activities. The obtained results are reported in the following table:

		Within Run	
Sample	**Mean (U/l)**	**SD**	**%CV**
Serum 1	49	0.7	1.43
Serum 2	186	2.8	1.50
Serum 3	301	5.2	1.72

Linearity
The assay is linear up to 800 U/l.

Sensitivity
Test sensitivity, in terms of limit of detection, is 2 U/l.

Correlation
A correlation study comparing the present method an a commercial one gave the following results:

y = 0.961x + 5.431 U/l r = 0.999

Interferences
Bilirubin > 20 mg/dl
Triglycerides > 10 g/l
A list of drugs and substances which cause changes in ALP levels or interfere with its measurement can be found in literature[2].

PRECAUTIONS IN USE

Refer to Safety Data Sheet.
Reagent A and Reagent B are not considered harmful according to 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.
The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management
Please refer to local legal requirements.

BIBLIOGRAPHY

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3. Tiets. N.W. Clinical Guide to Laboratory Tests 3th Edition. WB Saunders Co. Philadelphia, PA. (1995).
4. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC.
5. NCCLS Document, "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).

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