ALP
DGKC

Intended use:
In vitro test for the quantitative determination of alkaline phosphatase (ALP) in human serum and plasma.

Summary:
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. A rise in the alkaline phosphatase activity occurs with all forms of cholestasis, particularly with obstructive jaundice. It is also elevated in diseases of the skeletal system, such as Pagefs disease, hyperparathyroidism, rickets and osteomalacia, as well as with fractures and malignant tumors. A considerable rise in the alkaline phosphatase activity is sometimes seen in children and juveniles. It is caused by increased osteoblast activity following accelerated bone growth. Various reference values for the purposes of clinical evaluation have been assigned to different age groups.
In 1946, Bessey, Lowry and Brock published a method for the determination of alkaline phosphatase using p-nitrophenyl phosphate as substrate buffered with glycine/NaOH. In 1967, Hausamen et al improved upon the method by using diethanolamine as buffer. The "optimized standard method" by using diethanolamine as buffer. The assay described here meets the recommendations of the IFCC.

Test principle:
Colorimetric assay in accordance with a standardized method.

p - Nitrophenylphosphate+ H2O $\xrightarrow{\text{ALP, Mg2}}$ Phosphate + p - Nitrophenol

In the presence of magnesium and zinc ions, p-nitrophenyl phosphate is hydrolyzed by phosphatases to form phosphate and p-nitrophenol. In this process AMP serves as transient phosphate acceptor. The release of coloured p-nitrophenol is proportional to the ALP activity and can be measured photometrically.

Reagent concentration:

R1:	
AMP* buffer, pH 10.44 (30°C)	1,0 mmol/l
Magnesiumacetate	2,0 mmol/l
Zincsulfate	0,5 mmol/l
EDTA	2,0 mmol/l
R2:	
p – Nitrophenylphosphate	50 mmol/l

Preparation and stability:
Sample start:
4 Parts of R1 are mixed with one part R2. The resulting working reagent is stable:
60 daysat +2°C to +8°C
4 days at +20°C to +25°C

Substrate start/automated analyzer:
R1: Ready for use.
R2: Ready for use.
Unopened kit components: Up to the expiration date at +2°C to +8°C
Onboard stability:
R1: 28 days
R2: 28 days

Specimen:
Collect serum using standard sampling tubes.
Heparinized plasma.
Stability: 2 days at +20°C to +25°C
7 days at +4°C to + 8°C
4 weeks at -20°C
Centrifuge samples containing precipitate before performing the assay.

Limitations - interference:
Criterion: Recovery within ±10% of initial value.
Icterus: No significant interference up to an index I of 30 (approximate conjugated and unconjugated bilirubin: 30 mg/dl).
Hemolysis: No significant interference up to an index H of 200 (approximate hemoglobin concentration: 200 mg/dl).
Lipemia (Intralipid): No significant interference up to an index L of 1000 (approximate triglycerides concentration: 2000 mg/dl). There is poor correlation between turbidity and triglycerides concentration.

Testing procedure:
Applications for automated systems are available on request.
Materials provided
• Working solutions as described above
Additional materials required
• Calibrators and controls as indicated below 0.9% NaCl

Manual procedure for substrate start:	
Wavelength:	Hg 405 nm (400-420nm)
Temperature:	+37°C
Cuvette:	1 cm light path
Zero adjustment:	air or distilled water
Reagent 1	800 µl
Sample	20 µl
Reagent 2	200 µl
Mix, read initial absorbance and start stopwatch simultaneously. Read again after exactly 1, 2 and 3 minutes and calculate AA/min	
Calculation for substrate start: Formula valid with 1 cm cuvette, Hg 405 nm: A/min x 2757 = Activity (U/l)	

Measuring /reportable range:
Up to 3000 U/l or A/min > 0,500
Determine samples having higher activities via the rerun function. On instruments without rerun function, dilute the samples with 0.9% NaCl or distilled/deionized water manually (e.g. 1 + 4). Multiply the result by the appropriate dilution factor (e.g. factor 5).
Conversion factor: U/l x 0.0167 = µkat/

Expected values:

		+37°C
U/l	Men	< 270
	Women	< 240
µkat/l	Men	< 4,50
	Women	< 4,00

For reference values for children, please refer to „Pediatric reference ranges“ 3rd Edition. S.J. Soldin, C. Brugnara, I.M. Hicks, AACC Press.
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 alkaline phosphatase results should always be assessed in conjunction with the patients´ medical history, clinical examination and other findings.

Analytical sensitivity (lower detection limit)
Detection limit: 7 U/l
The lower detection limit represents the lowest measurable ALP concentration thatcan be distinguished from zero.

Imprecision:
Reproducibility was determined using human samples and controls within run (n =10). The following results were obtained:

	Within run		
Sample	Mean U/l	SD U/l	CV %
Sample 1	175,70	0,95	0,50
Sample 2	426	2,41	0,60

Reproducibility was determined using human samples and controls in an interna protocol within (between day n = 10). The following results were obtained:

	Between day		
Sample	Mean U/l	SD U/l	CV %
Sample 1	167,26	3,99	2,40
Sample 2	408,28	8,61	2,10

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Methods comparison:
a comparison between BIOANALYTIC and a commercially available product gave the following results
ALP Bıoanalytıc =x
ALP competitor=y n=112
y=0,96x – 2,17 U/l r^2=0,999

Quality Control:
Control Serum:

BIOCON N	5 x 5 ml	#B10814
BIOCON P	5 x 5 ml	#B10817

The control intervals and limits must be adapted to the individual laboratory and country-specific requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Calibration:
S1: 0.9% NaCl
S2: BIOCAL H 5 x 3 ml #B11895

Calibration frequency:
A two-point-calibration is recommended in case of:
1-change of lot
2- quality control requirements

Notes:
For in vitro diagnostic use.

Literature:
1. Bessey OAH et al. J Biol Chem 1946;164:321.
2. Empfehlungen der Deutschen Gesellschaft für Klinische Chemie. Standard-Methode zur Bestimmung der Aktivität der alkalischen Phosphatase. Z klin Chem u klin Biochem 1972;10:191.
3. Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-474.
4. Greiling H, Gressner AM (Hrsg.). Lehrbuch der Klinischen Chemie und Pathobiochemie, 3rd . Stuttgart/New York: Schattauer Verlag, 1995.
5. Guder WG, Narayanan S, Wisser H, Zawta B. List of Analytes Preanalytical Variables. Broschüre in: Samples: From the Patient to the Laboratory. Darmstadt: GIT Verlag,l996.
6. Hausamen TU et al. Clin Chim Acta 1967;15:241.
7. Rosalki SB, Foo AY, Burlina A et al. Multicenter Evaluation of Iso ALP Test Kit for Measurement of Bone Alkaline Phosphatase Activity in Serum and Plasma. Clin Chem 1993;39:648-652.

Order information (Cat No.) :

CC325	CR325	B27010	B32010	B36011
KL325	B21010	B27011	B32011	B37010
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SH325	B24010	B30010	B34010	B80011
BALP250	B25010	B30011	B35010	B80012
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SYMBOLS

IVD	for in vitro diagnostic use only
LOT	lot of manufacturing
REF	code number
	storage at temperature interval
	expiration date (year/month)
	warning, read enclosed documents
	Read the directions

ISO 9001:2015
ISO 13485:2016