ALKALINE PHOSPHATASE

Cat. No.	Pack Name	Packaging (Content)
BLT00003	ALP AMP 150	R1: 4 x 30 ml, R2: 1 x 30 ml
BLT00004	ALP AMP 500	R1: 4 x 100 ml, R2: 1 x 100 ml

EN

CE IVD

INTENDED USE
Diagnostic reagent for quantitative *in vitro* determination of ALP in human serum or plasma.

CLINICAL SIGNIFICANCE
Human ALP consists of a group of enzymes which hydrolyse phosphates at an alkaline pH. ALP is found in practically all tissues of the body but in high concentrations in the osteoblasts of bone, liver, placenta, kidney, intestinal wall and lactating mammary glands. In adults the ALP normally found circulating in the serum is largely derived from the liver. In children or in adolescents going through pubertal growth spurts, there is an additional contribution from bone and this accounts for the higher reference interval for these groups. Pregnancy also raises the normal values of ALP.
Raised ALP levels are often observed in bone disease or liver disease involving the biliary tract. If the source of the isoenzyme is not apparent then estimation of GGT may help differentiate between the two. A raised GGT in the presence of a raised ALP would suggest the liver is the primary source.
Increased ALP (usually normal GGT) is seen in Osteomalacia and Rickets, primary hyperparathyroidism with bone involvement, Pagets disease, secondary carcinoma in bone and some cases of osteogenic sarcoma. Increased levels of ALP (usually with a raised GGT) is seen in cholestasis, hepatitis, cirrhosis, space occupying lesions and malignancy with bone or liver involvement or direct production. Low levels of ALP may be observed in conditions which cause arrested bone growth or in hypophosphatasia.

PRINCIPLE
The method according to IFCC recommendation. This method utilises 4-nitrophenyl phosphate as the substrate. Under optimised conditions ALP present in the sample catalyses the following reaction.

$$AMP + 4\text{-}NPP + H_2O \xrightarrow[Mg_{2+}/\text{Alkaline pH}]{ALP} 4\text{-nitrophenol} + \text{phosphate}$$

At the pH of the reaction, 4-nitrophenol has an intense yellow colour. The reagent also contains a metal ion buffer system to ensure that optimal concentrations of Zinc and Magnesium are maintained. The metal ion buffer can also chelate other potentially inhibitory ions which may be present. The reaction is monitored by measuring the rate of increase in absorbance at 405 or 415 nm which is proportional to the activity of ALP in the serum.

REAGENT COMPOSITION
R1

AMP buffer, pH 10.4	434 mmol/l
Magnesium acetate	2.48 mmol/l
Zinc sulfate	1.24 mmol/l
HEDTA	2.48 mmol/l
R2	
p-nitrophenyl phosphate	19.5 mmol/l

REAGENT PREPARATION
Reagent is liquid, ready to use.

STABILITY AND STORAGE
The unopened reagents are stable till the expiry date stated on the bottle and kit label when stored at 2–8°C.
Two reagents method – substrate start
Reagents are ready to use. After opening, reagents are stable until expiry date at 2–8°C if stored at appropriate conditions, closed carefully and without any contamination.
Maximum allowable absorbance of the working reagent measured at 420 nm against distilled water is 1.0
Monoreagent method – sample start
Mix 4 portion of reagent R1 with 1 portion of reagent R2.
Stability: 1 week at 15–25°C in dark
4 weeks at 2–8°C in dark

SPECIMEN COLLECTION AND HANDLING
Use serum, plasma (heparin, EDTA).
It is recommended to follow NCCLS procedures (or similar standardized conditions).
Stability in serum / plasma: 4 hours at 20–25°C
3 days at 4–8°C
2 months at -20°C
Discard contaminated specimens.

CALIBRATION
Calibration with calibrator XL MULTICAL, Cat. No. XSYS0034 is recommended.

QUALITY CONTROL
For quality control ERBA NORM, Cat. No. BLT00080 and ERBA PATH, Cat. No. BLT00081 are recommended.

UNIT CONVERSION
U/l x 0.017 = µkat/l

EXPECTED VALUES [4]
at 37°C

Females:	4 - 15 years:	54 - 369 U/l
	20 - 50 years:	42 - 98 U/l
	≥ 60 years:	53 - 141 U/l
Males:	1 - 12 years:	54 - 369 U/l
	20 - 50 years:	53 - 128 U/l
	≥ 60 years:	56 - 119 U/l

It is recommended that each laboratory verify this range or derives reference interval for the population it serves.

PERFORMANCE DATA
Data contained within this section is representative of performance on ERBA XL systems. Data obtained in your laboratory may differ from these values.
Limit of quantification: 3.2 U/l
Linearity: 1080 U/l
Measuring range: 3.2 – 1080 U/l

Intra-assay precision Within run (n=20)	Mean (U/l)	SD (U/l)	CV (%)
Sample 1	297.6	2.58	0.87
Sample 2	460.8	4.92	1.07

Inter-assay precision Run to run (n=20)	Mean (U/l)	SD (U/l)	CV (%)
Sample 1	62.4	1.68	2.68
Sample 2	191.4	5.10	2.66

COMPARISON
A comparison between XL-Systems Amylase (y) and a commercially available test (x) using 40 samples gave following results:
y = 0.947 x - 3.60 U/l
r = 0.996

INTERFERENCES
Following substances do not interfere:
haemoglobin up to 5 g/l, bilirubin up to 40 mg/dl, triglycerides up to 2000 mg/dl.

WARNING AND PRECAUTIONS
For *in vitro* diagnostic use. To be handled by entitled and professionally educated person.
Reagents of the kit contain less than 0.1% sodium azide - classified as very toxic and dangerous substance for the environment.

Risk phrases (R):
R 36/38 Irritating to eyes and skin.
Safety phrases (S):
S 37/39 Wear suitable gloves and eye/face protection.
S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S 28 After contact with skin, wash immediately with plenty of water.
S 45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)

WASTE MANAGEMENT
Please refer to local legal requirements.

ASSAY PROCEDURE
Wavelength: 420 (405 – 430) nm
Cuvette: 1 cm
Two reagents method - substrate start

	Reagent blank	Calibrator	Sample
Reagent 1	0.800 ml	0.800 ml	0.800 ml
Sample	-	-	0.020 ml
Calibrator	-	0.020 ml	-
Distilled water	0.020 ml	-	-

Mix and after 5 min. incubation (at 37°C) add:

Reagent 2	0.200 ml	0.200 ml	0.200 ml

Mix, incubate 1 min. at 37°C and then measure the initial absorbance of calibrator and sample against reagent blank. Measure the absorbance change exactly after 1, 2 and 3 min. Calculate 1 minute absorbance change (ΔA/min).

Monoreagent method - sample start

	Reagent blank	Calibrator	Sample
Working reagent	1.000 ml	1.000 ml	1.000 ml
Sample	-	-	0.020 ml
Calibrator	-	0.020 ml	-
Distilled water	0.020 ml	-	-

Mix, incubate 1 min. at 37°C and then measure the initial absorbance of calibrator and sample against reagent blank. Measure the absorbance change exactly after 1, 2 and 3 min. Calculate 1 minute absorbance change (ΔA/min).

CALCULATION
1. $ALP\ (U/l) = \frac{\Delta A_{sam}/min.}{\Delta A_{cal}/min.} \times C_{cal}$ C_{cal} = calibrator concentration
2. Using factor:
ALP (U/l) = f x ΔA/min
f = factor f = 2764 (at 405 nm)

Applications for automatic analysers are available on request.

ASSAY PARAMETERS FOR PHOTOMETERS

Mode	Kinetic
Wavelength (nm)	405
Sample Volume (µl)	10/20
Working Reagent Volume (µl)	500/1000
Lag time (sec.)	60
Kinetic interval (sec.)	60
No. of readings	3
Kinetic factor	2764
Reaction temperature (°C)	37
Reaction direction	Increasing
Normal Low U/l	42
Normal High U/l	128
Linearity Low U/l	3.2
Linearity High U/l	1080
Blank with	Reagent
Absorbance limit (max.)	1.4
Units	U/l

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REFERENCES

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SYMBOLS USED ON LABELS

REF Catalogue Number

Manufacturer

See Instruction for Use

LOT Lot Number

CE Mark - Device comply with the Directive 98/79/EC

Storage Temperature

Expiry Date

IVD In Vitro Diagnostics

CONT Content

QUALITY SYSTEM CERTIFIED
ISO 9001 ISO 13485

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