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ALKALINE PHOSPHATASE

CONCENTRATED LIQUID REAGENT

CE

Order information:

Catalogue number	Size
9451C	10 x 65 + 10 x 17 ml
9452C	4 x 65 + 4 x 17 ml

Reagent kit for quantitative in vitro determination of alkaline phosphatase in serum and plasma.

Summary

Alkaline phosphatase is a membrane-bound enzyme which is present in most tissues. It has three different isoenzymes derived from small intestine-placenta-bone/liver/kidney. It is a dimer molecule containing Zn^{++} ions, which play a role in the maintenance of structure and catalysis. The enzyme found in human serum is derived from bone, liver and small intestine. During pregnancy the enzyme from the placenta dominates (it is heat stable at 65°C). In the past the isoenzymes were separated using various inhibitors and heat. The role of electrophoresis is growing in determining the concentrations. The increase in enzyme activity is prevalent in various hepatic and bone decrease states. The level is also increased in certain diseases of the thyroid gland, intestinal tract and in several bacterial infection.

Method

Kinetic, photometric, optimized DGKC mehtod.

$$\text{p-nitrophenylphosphate} + H_2O \xrightarrow{ALP/Mg^{2+}} \text{p-nitrophenol} + \text{phosphate}$$

Reagents

Composition and concentrations

Reagent 1

Dietanolamine buffer (pH= 9,80)	3,0 mol/l
Magnesium-chloride	1,8 mmol/l

Reagent 2

p-Nitrophenyl-phosphate	30,0 mmol/l

Storage and stability

The reagent is stable up to the end of the indicated month of expiry without opening, if stored at 2 – 8°C, protected from light and contamination is avoided. Do not freeze!
Onboard stability after opening and the frequeny of calibration is 50 days.
The absorbance at 410 nm should not be higher than 1,5

Warnings and precautions

Do not use reagents after the expiry date stated on each reagent container label.

Chemical safety

Reagent 1

X, Harmful
R22 Harmful if swallowed
R38 Irritating to skin.
R41 Risk of serious damage to eyes
R48/22 Harmful: danger of serious damage to health by prolonged exposure if swallowed.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37/39 Wear suitable protective clothing, gloves and eye/face protection
S46 If swallowed, seek medical advice immediately and show this container or label.

Preparation

The reagent is ready for use.

Sample

Serum, heparin, citrate or EDTA plasma.

Stability in serum:	2 – 3 days at 0 – 4°C
	1 month at -25°C

Expected values and reference range

Serum: 80 – 244 U/l
It is recommended that each laboratory should assign its own normal range.

Assay procedure

Wavelength:	410 nm / 505 nm (primary/secondary)
Optical path:	1 cm
Temperature:	37°C
Measurement:	against reagent blank
Reaction:	kinetic, increasing

	blank	sample or standard
reagent 1	240 µl	240 µl
dist. water (diluent)	480 µl	480 µl
dist. water (blank)	18 µl	-
sample or standard	-	18 µl
Mix and incubate for 1 minute		
reagent 2	60 µl	60 µl
dist. water (diluent)	120 µl	120 µl
Mix and incubate for 1 minute then continuously read the absorbances for 2 minutes		

Calculation

Alkaline phosphatase[U/l]=ΔA sample/ΔA standard × standard concentration[U/l]

Conversion factor

[U/l]=[µkat/l]×60

Calibration and quality control

S1: Distilled water
S2: Roche C.F.A.S. (Calibrator for automated system) or
Randox Calibration Serum Level I or
Randox Calibration Serum Level II
Calibration is recommended:

- after opening new reagent batch
- after system maitenance or troubleshooting

For internal quality control, two levels controls are recommended (normal and pathological) at least once a day. The measured values must in the range which was given by the control's manufacturer. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Preformance characteristics

Measuring range

The method is linear in the range 35 – 2100 U/l

Interferences

No significant intereference was observed by bilirubin up to 1000 µmol/l bilirubin, triglycerides up to 12 mmol/l, hemoglobin up to 0,8 g/l and ascorbate up to 4 g/l. Significant interference: >10%.

Limit of detection

The limit of detection is 0,001 U/l

Precision

Repeatability	mean	SD	CV
n = 20	[U/l]	[U/l]	[%]
normal sample	267	0,88	0,33
pathological sample	455	1,46	0,32
Reproduceability	**mean**	**SD**	**CV**
n = 10	[U/l]	[U/l]	[%]
normal sample	265	9,94	3,75
pathological sample	451	13,9	3,08

Method comparison

Comparison with the non-concentrated reagent.
analyser: Advia 1650
number of samples: 179
range: 64 – 2618 U/l
correlation coefficient: 0,9984
regression line equation: y = 0,902x – 7,573
(x= normal reagent, y= concentrated reagent)

The following symbols can be used on the labels

IVD	In vitro diagnostic device
	Manufacturer
CE	CE-marking
	Temperature limitations
	Use by (year/month)
LOT	Batch code
REF	Catalogue number
	This way up
	Biological risk

Literature

HaussamentT.U. et a L Clin. Chim. Act. 35,271-273,(1977)
Tietz Clinical Guide To Laboratory Tests, 4th edition, Elsevier, 2006