Alkaline Phosphatase FS

Alkaline Phosphatase Reagent Test Kit

Intended Use

Diagnostic reagent for in vitro quantitative determination of Alkaline Phosphatase in human serum or plasma on photometric analyzers.

Order Information

Item Code	Pack Size
104419934840	R1 : 4 x 60 mL; R2 : 4 x 15 mL

Summary [1,2]

Alkaline phosphatase (AP), a hydrolytic enzyme acting optimally at alkaline pH, exists in blood in numerous distinct forms which originate mainly from bone and liver, but also from other tissues as kidney, placenta, testes, thymus, lung and tumors. Physiological increases are found during bone growth in childhood and in pregnancy, while pathological increases are largely associated with hepatobiliary and bone diseases. In hepatobiliary disease they indicate obstruction of the bile ducts as in cholestasis caused by gall stones, tumors or inflammation. Elevated activities are also observed in infectious hepatitis. In bone diseases elevated AP activities originate from increased osteoblastic activity as in Paget's disease, osteomalacia (rickets), bone metastases and hyperparathyroidism.

Method

Kinetic photometric test, according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)

Principle

p-Nitrophenylphosphate + H_2O $\xrightarrow{AP}$ Phosphate + p-Nitrophenol

Reagents

Components and Concentrations

R1:	2 amino Methyl Propanol	36ml/L
	Zinc sulphate	0.4g/L
	Magnesium acetate	0.6g/L
R2:	p-Nitro Phenyl Phosphate	21g/L
	Preservatives & Stabilizer	q.s.

Storage Instructions and Reagent Stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C and contamination is avoided. Do not freeze the reagents!
Reagent 2 must be protected from light.

Waste Management

Please refer to local legal requirements.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment
TruCal U(591009910064)
TruLab N(590009910061)
TruLab P(590509910061)

Warnings and Precautions

1. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. During reaction p-nitrophenol is produced which is poisonous when inhaled, swallowed or absorbed through skin. If the reaction mixture comes in contact with skin or mucous membranes wash copiously with water!
3. In very rare cases, samples of patients with gammopathy might give falsified results [9].
4. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
5. For professional use only!

Reagent Preparation

The reagents are ready to use.

Traceability

This method is traceable to the molar extinction coefficient.

Specimen

Serum or heparin plasma
Do not use hemolytic samples!

Stability [4]:	7 days	at	20 – 25°C
	7 days	at	4 – 8°C
	2 months	at	–20°C

Only freeze once! Discard contaminated specimens!

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength	405 nm, (400 – 420 nm)
Optical path	1 cm
Temperature	37°C
Measurement	Against reagent blank

	Blank	Sample or calibrator
Sample or calibrator	-	20 µL
Dist. Water	20 µL	-
Reagent 1	1000 µL	1000 µL
Mix, incubate for approx. 1 min., then add:		
Reagent 2	250 µL	250 µL
Mix, read absorbance after 1 min. and start stopwatch. Read absorbance again after 1, 2 and 3 min.		

Calculation

With factor

From absorbance readings calculate A/min and multiply by the corresponding factor from table below:

ΔA/min x factor = AP activity [U/L]

405 nm	3433

With calibrator

$$AP\ [U/L] = \Delta\frac{\Delta A/min\ Sample}{\Delta A/min\ Calibrator} \times Conc.\ Calibrator\ [U/L]$$

Calculation factor

ALP [U/L] x 0.0167 = ALP [µkat/L]

Calibrators and Controls

For the calibration of automated photometric systems, DiaSys TruCal U calibrator is recommended. This method is traceable to the molar extinction coefficient. For internal quality control DiaSys TruLab N and P controls should be assayed. Each laboratory should establish corrective action in case of deviations in control recovery.

Performance characteristics

Measuring range

On automated systems the test is suitable for the determination of AP activities up to 1400 U/L.
In case of a manual procedure, the test is suitable for AP activities which correspond to a maximum of ΔA/min of 0.25.
If such values are exceeded the samples should be diluted 1 + 9 with NaCl solution (9 g/L) and results multiplied by 10.

Specificity/Interferences

No interference was observed by ascorbic acid up to 30 mg/dL, conjugated bilirubin up to 60 mg/dL, unconjugated bilirubin to 25 mg/dL, hemoglobin up to 100 mg/dL and lipemia up to 2000 mg/dL triglycerides. For further information on interfering substances refer to Young DS [5].

Sensitivity/Limit of Detection

The lower limit of detection is 2 U/L.

Precision

Intra-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	71.88	1.15	1.60
Sample 2	162.5	1.24	0.77
Sample 3	387.8	1.38	1.39

Inter-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	86.2	1.53	1.78
Sample 2	121	0.79	0.65
Sample 3	471	1.21	1.11

Method Comparison

A comparison of DiaSys Alkaline phosphatase (y) with a commercially available test (x) using 104 samples gave following results: y = 1.01 x – 1.51 U/L; r = 0.999

Clinical Intepretation

Adults [6]

Women	35 – 104 [U/L]	0.58 – 1.74 µkat/L
Men	40 – 129 [U/L]	0.67 – 2.15 µkat/L

Adults [7]

Women	35 – 105 [U/L]	0.58 – 1.75 µkat/L
Men	40 – 130 [U/L]	0.67 – 2.17 µkat/L

Children [8]

	Female [U/L]	Male [U/L]	Female [µkat/L]	Male [µkat/L]
1 – 30 day(s)	48 – 406	75 – 316	0.80 – 6.77	1.25 – 5.27
1 month – 1 year	124 – 341	82 – 383	2.07 – 5.68	1.37 – 6.38
1 – 3 year(s)	108 – 317	104 – 345	1.80 – 5.28	1.73 – 5.75
4 – 6 years	96 – 297	93 – 309	1.60 – 4.95	1.55 – 5.15
7 – 9 years	69 – 325	86 – 315	1.15 – 5.42	1.43 – 5.25
10 – 12 years	51 – 332	42 – 362	0.85 – 5.53	0.70 – 6.03
13 – 15 years	50 – 162	74 – 390	0.83 – 2.70	1.23 – 6.50
16 – 18 years	47 – 119	52 – 171	0.78 – 1.98	0.87 – 2.85

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Limitations

Result interference can be seen in the samples where triglyceride conc. Exceeds 2000 mg/dL.

Literature

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5. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001; p. 14-5.
6. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
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9. Soldin JS, Brugnara C., Wong CE. In: MJ Hicks, editor. Pediatric reference intervals. 6th ed. Washington: AACC Press, 2007. p. 11.
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Notes on Symbols and Marks

	Consult instruction for use
	Use-by date
LOT	Batch code
REF	Catalogue number
	Caution
	Manufacturer
IVD	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
CONT.	The pack contains
	Recycle
	Date of manufacture

ISO 9001, ISO 13485 and ICMED 13485 Certified Company

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