Alkaline phosphatase FS*

IFCC mod. 37°C

Order Information

Cat. No.	Kit size				
1 0441 99 10 021	R1	5 x 20 mL	+	R2	1 x 25 mL
1 0441 99 10 026	R1	5 x 80 mL	+	R2	1 x 100 mL
1 0441 99 10 023	R1	1 x 800 mL	+	R2	1 x 200 mL
1 0441 99 10 704	R1	8 x 50 mL	+	R2	8 x 12.5 mL
1 0441 99 10 917	R1	8 x 60 mL	+	R2	8 x 15 mL
1 0441 99 10 930	R1	4 x 20 mL	+	R2	2 x 10 mL
1 0441 99 90 314	R1	10 x 20 mL	+	R2	2 x 30 mL

Intended Use

Diagnostic reagent for quantitative in vitro determination of alkaline phosphatase (AP) in serum or plasma on photometric systems.

Summary

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. [1,2]

Method

Kinetic photometric test, according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) [modif.] [3].

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

Reagents

Components and Concentrations

R1:	2-Amino-2-methyl-1-propanol	pH 10.4	1.1 mol/L
	Magnesium acetate		2 mmol/L
	Zinc sulphate		0.5 mmol/L
	HEDTA		2.5 mmol/L
R2:	p-Nitrophenylphosphate		80 mmol/L

Storage and 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 and protect them from light.

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 the 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 [4].
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.

Waste Management

Refer to local legal requirements.

Reagent Preparation

Substrate Start

The reagents are ready to use.

Sample Start

Mix 4 parts of R1 + 1 part of R2
(e.g. 20 mL R1 + 5 mL R2) = mono reagent

Stability:			
	4 weeks	at	2 – 8°C
	5 days	at	15 – 25°C

The mono reagent must be protected from light.

Materials Required

General laboratory equipment

Specimen

Serum or heparin plasma

Do not use hemolytic samples.

Stability [5]:

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

Applications for automated systems are available on request.

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

Substrate Start

	Blank	Sample/calibrator
Sample/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.		

Sample Start

	Blank	Sample/calibrator
Sample/calibrator	-	20 µL
Dist. Water	20 µL	-
Mono reagent	1000 µL	1000 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 Δ A/min and multiply by the corresponding factor from table below:

Δ A/min x factor = AP activity [U/L]

Substrate start	405 nm	3433
Sample start	405 nm	2757

With calibrator

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

Conversion Factor

AP [U/L] x 0.0167 = AP [µkat/L]

Calibrators and Controls

DiaSys TruCal U is recommended for calibration. This method is traceable to the molar extinction coefficient. Use DiaSys TruLab N and P for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
TruCal U	5 9100 99 10 063	20 x 3 mL
	5 9100 99 10 064	6 x 3 mL
TruLab N	5 9000 99 10 062	20 x 5 mL
	5 9000 99 10 061	6 x 5 mL
TruLab P	5 9050 99 10 062	20 x 5 mL
	5 9050 99 10 061	6 x 5 mL

Performance Characteristics

Data evaluated on BioMajesty® JCA-BM6010/C

Exemplary data mentioned below may slightly differ in case of deviating measurement conditions.

Measuring range 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.	
Limit of detection**	0.6 U/L
Onboard stability	6 days
Calibration stability	6 days

Interfering substance	**Interferences ≤ 10% up to**
Ascorbic acid	30 mg/dL
Bilirubin (conjugated)	60 mg/dL
Bilirubin (unconjugated)	36 mg/dL
Hemoglobin	150 mg/dL
Lipemia (triglycerides)	2000 mg/dL
For further information on interfering substances refer to Young DS [6].	

Precision			
Within run (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [U/L]	86.4	197	277
CV [%]	0.66	0.72	0.53
Between day (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [U/L]	29.7	139	305
CV [%]	3.10	1.49	1.70

Method comparison (n=100)	
Test x	Competitor Alkaline Phosphatase (AP)
Test y	DiaSys Alkaline Phosphatase FS
Slope	1.03
Intercept	3.96 U/L
Coefficient of correlation	0.9998

** lowest measurable activity which can be distinguished from zero; mean + 3 SD (n = 20) of an analyte free specimen.

Reference Range

Adults [7]		
Women	35 – 104 [U/L]	0.58 – 1.74 µkat/L
Men	40 – 129 [U/L]	0.67 – 2.15 µkat/L

Adults [8]		
Women	35 – 105 [U/L]	0.58 – 1.75 µkat/L
Men	40 – 130 [U/L]	0.67 – 2.17 µkat/L

Children [9]				
	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.

Literature

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4. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007;45(9):1240-1243.
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* Fluid Stable