Alkaline phosphatase FS*

DGKC

Order Information

Cat. No.	Kit size				
1 0401 99 10 021	R1	5 x 20 mL	+	R2	1 x 25 mL
1 0401 99 10 026	R1	5 x 80 mL	+	R2	1 x 100 mL
1 0401 99 10 023	R1	1 x 800 mL	+	R2	1 x 200 mL
1 0401 99 10 704	R1	8 x 50 mL	+	R2	8 x 12.5 mL
1 0401 99 10 930	R1	4 x 20 mL	+	R2	2 x 10 mL
1 0401 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, optimized standard method according to the German Society of Clinical Chemistry (DGKC) [3].

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

Reagents

Components and Concentrations

R1:	Diethanolamine	pH 9.8	1.2 mol/L
	Magnesium chloride		0.6 mmol/L
R2:	p-Nitrophenylphosphate		50 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. ⚠ Reagent 1: Danger. Contains Diethanolamine. H315 Causes skin irritation. H318 Causes serious eye damage. H373 May cause damage to organs through prolonged or repeated exposure. P260 Do not breathe vapors. P280 Wear protective gloves/protective clothing/eye protection. P302+P352 If on skin: Wash with plenty of water/soap. P305+P351+P338 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P310 Immediately call a poison centre/doctor.
2. Reagent 2 contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
3. 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!
4. In very rare cases, samples of patients with gammopathy might give falsified results [4].
5. 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.
6. 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

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	25°C/30°C/37°C
Measurement	Against air

Substrate Start

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

Sample Start

Sample or calibrator	20 µL
Mono reagent	1000 µL

Mix, read absorbance after 1 min. and start stopwatch. Read absorbance again after 1, 2 and 3 min.

Calculation

With factor

Calculate ΔA/min from absorbance readings and multiply by the corresponding factor from table below:

ΔA/min x factor = AP activity [U/L]

Substrate start	405 nm	3433
Sample start	405 nm	2757

With calibrator

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

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

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

Measuring range up to 4500 U/L.
In case of a manual procedure, the test is suitable for alkaline phosphatase activities which correspond to a maximum of Δ A / min of 0.25.

When values exceed this range samples should be diluted 1 + 9 with NaCl solution (9 g/L) and the result multiplied by 10.

Limit of detection**	3 U/L

Interfering substance	**Interferences ≤ 10% up to**
Ascorbic acid	30 mg/dL
Bilirubin	40 mg/dL
Hemoglobin	150 mg/dL
Lipemia (triglycerides)	2000 mg/dL

For further information on interfering substances refer to Young DS [6].

Precision (at 25°C)			
Within run (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [U/L]	114	222	275
CV [%]	1.50	0.92	1.06
Between day (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [U/L]	120	223	279
CV [%]	1.60	0.85	0.85

Method comparison (n=78)	
Test x	Competitor alkaline phosphatase (AP)
Test y	DiaSys Alkaline phosphatase FS DGKC
Slope	0.979
Intercept	–2.21 U/L
Coefficient of correlation	r = 0.999

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

Reference Range

As follows: [7]

		25°C	30°C	37°C
Children 1 – 12 year(s)	[U/L]	< 480	< 596	< 727
	[µkat/L]	< 8.00	< 9.93	< 12.1
Female 13 –17 years	[U/L]	< 296	< 367	< 448
	[µkat/L]	< 4.93	< 6.12	< 7.47
Male 13 –17 years	[U/L]	< 617	< 767	< 935
	[µkat/L]	< 10.3	< 12.8	< 15.6
Adults	[U/L]	< 170	< 211	< 258
	[µkat/L]	< 2.83	< 3.52	< 4.30

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