ISE S.r.l.

CUSTOMISED SOLUTIONS
FOR YOUR LABORATORY

ALP DEA – Instructions for use

CE

IVD

REF

R1: 2x 18 mL R2: 2x 4,5 mL **R3330000006**

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

TEST PARAMETERS

Method:	Colorimetric, kinetic, increasing reaction, optimized DGKC
Wavelength:	405 nm (400 – 420 nm)
Temperature:	37°C
Sample:	Serum, heparin plasma
Linearity:	up to 800 U/L (manual test procedure) up to 4500 U/L (automatic test procedure)
Sensitivity:	The lower limit of detection is 3 U/L

SUMMARY [1,2]

Alkaline Phosphatase (ALP), 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 tumours. 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, tumours or inflammation. Elevated activities are also observed in infectious hepatitis. In bone diseases elevated ALP activities originate from increased osteoblastic activity as in Paget's disease, osteomalacia (rickets), bone metastases and hyperparathyroidism.

TEST PRINCIPLE

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

Under alkaline condition, colorless p-nitrophenol is converted to 4-nitrophenoxide, which develops a very intense yellow color. Increase of absorbance is proportional to the activity of alkaline phosphatase in the sample.

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION
Reagent 1:	
Diethanolamine, pH 9.8	1.2 mol/L
Magnesium chloride	0.6 mmol/L
Reagent 2:	
p-Nitrophenylphosphate	50 mmol/L

REAGENT PREPARATION

Substrate Start:
The reagents are ready to use.
Sample Start:
Mix 4 parts of Reagent 1 with 1 part of Reagent 2
(= Working Reagent)

REAGENT STABILITY AND STORAGE

Conditions:	Protect from light. Close immediately after use. Avoid contamination. Do not freeze the reagents!
Substrate Start:	
Storage:	at 2 – 8 °C
Stability:	up to the expiration date indicated on labels

Sample Start (Working Reagent):

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

The working reagent must be protected from light!

SAMPLE STABILITY AND STORAGE

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

Discard contaminated specimens!
Freeze only once!

MATERIALS REQUIRED BUT NOT PROVIDED

NaCl solution (9 g/L)
General laboratory equipment

MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.

Substrate Start

Pipette into test tubes	37°C
Reagent 1	1000 µL
Sample	20 µL
Mix. Incubate for approximately 1 minute. Then add:	
Reagent 2	250 µL
Mix. Read initial absorbance against air after 1 minute and start a stopwatch. Read absorbance again after exactly 1, 2 and 3 min. Determine ΔA/min. during the linear part of the assay.	

Sample Start

Pipette into test tubes	37°C
Working Reagent	1000 µL
Sample	20 µL
Mix. Read initial absorbance against air after 1 minute and start a stopwatch. Read absorbance again after exactly 1, 2 and 3 min. Determine ΔA/min. during the linear part of the assay.	

CALCULATION

With factor: (light path 1 cm)

Alkaline Phosphatase [U/L] = ΔA/min x Factor

Factors (405 nm, 37°C): Substrate Start: 3433
Sample Start: 2757

With calibrator:

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

UNIT CONVERSION

U/L x 0,01667 = µkat/L

REFERENCE RANGES [6] * (37 °C)

	Year(s)	U/L	µkat/L
Adults:		< 258	< 4.30
Children:	1–12 year(s)	< 727	< 12.1
Females	13-17 years	< 448	< 7.47
Males	13-17 years	< 935	< 15.6

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

PERFORMANCE CHARACTERISTICS

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LINEARITY, MEASURING RANGE

On automated systems the test is suitable to determine alkaline phosphatase activities up to 4500 U/L. In case of a manual procedure, the test is suitable for alkaline phosphatase activities up to 800 U/L which correspond to a maximum ΔA/min of 0.25. If the value is exceeded, the sample should be diluted 1+ 9 with NaCl solution (9 g/L) and the result multiplied by 10.

SENSITIVITY/LIMIT OF DETECTION

The lower limit of detection is 3 U/L

PRECISION

Intra-assay n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	114	1.71	1.50
Sample 2	222	2.05	0.92
Sample 3	275	2.91	1.06

Inter-assay n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	120	1.93	1.60
Sample 2	223	1.89	0.85
Sample 3	279	2.36	0.85

SPECIFICITY/INTERFERENCES

no interference up to:

Ascorbic acid	30 mg/dL
Bilirubin	40 mg/dL
Hemoglobin	150 mg/dL
Triglycerides	2000 mg/dL

For further information on interfering substances refer to Young DS [5].

METHOD COMPARISON

A comparison of this Alkaline phosphatase opt. DGKC (y) with a commercially available test (x) using 78 samples gave following results:
y = 0.98 x – 2.21 U/L; r= 0.999.

CALIBRATION

The use of an Alkaline Phosphatase Calibrator is optional. We recommend our multicalibrator which method is traceable to the molar extinction coefficient.

QUALITY CONTROL

All control sera with Alkaline Phosphatase values determined by this method can be used. We recommend our serum controls Normal control serum (control serum with values in the normal range) and Pathological control serum (control serum with values in the abnormal range). Each laboratory should establish corrective action in case of deviations in control recovery.

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WARNINGS AND PRECAUTIONS

1. Reagent 1: Danger.
   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/face 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 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! In very rare cases, samples of patients with gammopathy might give falsified results [7].
4. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
5. 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

Please refer to local legal requirements

REFERENCES

1. Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 36-46.
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3. Deutsche Gesellschaft für klinische Chemie. Empfehlungen der deutschen Gesellschaft für Klinische Chemie (DGKC). Standardisierung von Methoden zur Bestimmung von Enzymaktivitäten in biologischen Flüssigkeiten. (Recommendation of the German Society of Clinical Chemistry. Standardization of methods for measurement of enzymatic activities in biological fluids.) Z Klin Chem Klin Biochem 1972;10:182-92.
4. Guder WG, Zawta B et al. The quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001; p. 14-5.
5. 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.
6. Fischbach F, Zawta B. Age-dependent reference limits of several enzymes in plasma at different measuring temperatures. Klin Lab 1992;38:555-61.
7. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007; 45(9): 1240-1243.

Symbols on labels and packaging

IVD = In vitro diagnostic medical device

REF = Catalog Number

LOT = Lot Number

= Manufacturer

= Expiration date

= Temperature limitation

= Instruction for use

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