COD 11590 50 mL	COD 11591 200 mL	COD 11597 500 mL
STORE AT 2-8ºC		
Reagents for measurement of ALP concentration Only for *in vitro* use in the clinical laboratory		

ALKALINE PHOSPHATASE (ALP) - DEA

BioSystems REAGENTS & INSTRUMENTS

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ALKALINE PHOSPHATASE (ALP) - DEA
DIETHANOLAMINE BUFFER

PRINCIPLE OF THE METHOD

Alkaline phosphatase (ALP) catalyzes in alkaline medium the transfer of the phosphate group from 4-nitrophenylphosphate to diethanolamine (DEA), liberating 4-nitrophenol. The catalytic concentration is determined from the rate of 4-nitrophenol formation, measured at 405 nm[1,2].

$$\text{4-nitrophenylphosphate + DEA} \xrightarrow{\text{ALP}} \text{DEA - phosphate + 4-Nitrophenol}$$

CONTENTS

	COD 11590	COD 11591	COD 11597
A. Reagent	1 x 40 mL	4 x 40 mL	4 x 100 mL
B. Reagent	1 x 10 mL	4 x 10 mL	2 x 50 mL

COMPOSITION

A. Reagent: Diethanolamine 1.2 mol/L, magnesium chloride 0.6 mmol/L, pH 9.8.

WARNING: H302: Harmful if swallowed. P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell. P330: Rinse mouth.

B. Reagent: 4-Nitrophenylphosphate 60 mmol/L.

For further warnings and precautions, see the product safety data sheet (SDS).

STORAGE

Store at 2-8ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagents: Presence of particulate material, turbidity, absorbance of the blank over 1.200 at 405 nm (1 cm cuvette).

REAGENT PREPARATION

Working Reagent:

- Cod. 11590 and 11591: Transfer the contents of one Reagent B vial into a Reagent A bottle. Mix gently. Other volumes can be prepared in the proportion: 4 mL Reagent A + 1 mL Reagent B. Stable for 2 months at 2-8ºC.
- Cod. 11597 : Transfer 25 mL of one Reagent B vial into a Reagent A bottle. Mix gently. Other volumes can be prepared in the proportion: 4 mL Reagent A + 1 mL Reagent B. Stable for 2 months at 2-8ºC.

ADDITIONAL EQUIPMENT

– Analyzer, spectrophotometer or photometer with cell holder thermostatable at 25, 30 or 37ºC and able to read at 405 nm.

– Cuvettes with 1 cm light path.

SAMPLES

Serum and plasma collected by standard procedures.

Alkaline phosphatase in serum or plasma is stable for 7 days at 2-8ºC. Heparin may be used as anticoagulant.

PROCEDURE

1. Bring the Working Reagent and the instrument to reaction temperature.
2. Pipette into a cuvette: (Note 1)

Working Reagent	1.0 mL
Sample/Calibrator	20 µL

3. Mix and insert the cuvette into the photometer. Start the stopwatch.
4. Record initial absorbance and at 1 minute intervals thereafter for 3 minutes.
5. Calculate the difference between consecutive absorbances, and the average absorbance difference per minute (ΔA/min).

CALCULATIONS

The ALP concentration in the sample is calculated using the following general formula:

$$\Delta A/\text{min} \times \frac{Vt \times 10^6}{\varepsilon \times l \times Vs} = U/L$$

The molar absorbance (ε) of 4-nitrophenol at 405 nm is 18450, the lightpath (l) is 1 cm, the total reaction volume (Vt) is 1.02, the sample volume (Vs) is 0.02, and 1 U/L are 0.0166 µkat/L. The following formulas are deduced for the calculation of the catalytic concentration:

ΔA/min	x 2764 = U/L x 46.08 = µkat/L

REFERENCE VALUES

Reaction temperature	men[3]	women[3]
25ºC, up to	180 U/L = 3.00 µKat/L	160 U/L = 2.67 µKat/L
30ºC, up to	220 U/L = 3.67 µKat/L	195 U/L = 3.25 µKat/L
37ºC, up to	270 U/L = 4.50 µKat/L	240 U/L = 4.00 µKat/L

Concentrations in growing children are higher and highly variable.These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

– Detection limit: 1.6 U/L = 0.027 µkat/L.

– Linearity limit: 900 U/L = 15.0 µkat/L. For higher values dilute sample 1/2 with distilled water and repeat measurement.

– Repeatibility (within run):

Mean Concentration	CV	n
117 U/L = 1.95 µkat/L	1.1 %	20
431 U/L = 7.18 µkat/L	0.7 %	20

– Reproducibility (run to run):

Mean Concentration	CV	n
117 U/L = 1.95 µkat/L	4.5 %	25
431 U/L = 7.18 µkat/L	2.2 %	25

– Sensitivity: 0.362 ΔmA·L/U·min = 0.022 ΔmA·L/µkat·min

– Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

– Interferences: Lipemia (triglycerides < 10 g/L) and bilirubin (< 20 mg/dL) do not interfere. Hemoglobin (> 5 g/L) interfere. Other drugs and substances may interfere[4].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Alkaline phosphatase catalyzes the hydrolysis of organic phosphate monoesters at alkaline pH. The enzyme is present in practically all tissues of the body, especially at or in the cell membranes, and it occurs at particularly high concentrations in placenta, intestinal epithelium, kidney tubules, osteoblasts and liver.

The form present in the sera of normal adults originates mainly in the liver and bone.

Elevated serum ALP is found in patients with bone disease associated with increased osteoblastic activity (Paget's disease, primary and secondary hyperparathyroidism, bone tumors, rickets, osteomalacia, bone fractures) and also in patients with hepatobiliary disease (obstructive jaundice, hepatitis, hepatotoxicity caused by drugs, liver cancer). Physiological changes, such as bone growth and pregnancy, may cause increases in ALP levels[5,6].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.

BIBLIOGRAPHY

1. The Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology. Recommended methods for determination of four enzymes in blood. *Scand J Clin Lab Invest* 1974; 33:291-306.
2. Empfehlungen der Deutschen Gesellschaft für Klinische Chemie. Standardisierung von methoden zur bestimmung von enzymaktivitäten in biologischen flüssigkeiten. *Z Klin Chem Klin Biochem* 1970; 8:658-660.
3. Rosalki SB, Foo AY, Burlina A, at al. Multicenter evaluation of iso-ALP test kit for measurement of bone alkaline phosphatase activity in serum and plasma. Clin Chem 1993; 39:648-652.
4. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
5. Friedman and Young. Effects of disease on clinical laboratory tests, 4th ed. AACC Press, 2001.
6. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.