N BIO - ALKALINE PHOSPHATASE

(pNPP - AMP Buffer method)

KIT NAME	KIT SIZE	CAT. NO
N BIO - ALP	2 X 50 ml	DALP02050M

GENUINE BIOSYSTEM

INTRODUCTION

Alkaline phosphatase (ALP) is actually a group of isoenzymes that hydrolyse monophosphate esters in alkaline medium. Optimum pH for these ALP isoforms activities is about 9-10. Alkaline phosphatase level is the highest in liver, bone, intestine, kidney and placenta. Measurement of ALP isoenzymes is useful in diagnosis of these organs diseases.

METHOD PRINCIPLE

Kinetic method recommended by International Federation of Clinical Chemistry (IFCC).

2-amino-2-methyl-1-propanol + p-nitrophenylophosphate + H2O
ALP> 4-nitrophenol + 2-amino-2-methyl-1-propanol phosphate

The rate of 4-nitrophenol formation is directly proportional to the ALP activity.

KIT CONTENTS

Reagent name	DALP02050M
R1 - ALP reagent	2 x 40 ml
R2 - ALP reagent	2 x 10 ml

The reagents when stored at 2-8°C are stable up to expiry date printed on the package. The reagents are stable for 10 days on board the analyser at 2-10°C. Protect from light and avoid contamination.

WORKING REAGENT PREPARATION AND STABILITY

Assay can be performed with use of separate R1-ALP and R2-ALP reagents or with use of working reagent. For working reagent preparation mix gently 4 parts of R1-ALP with 1 part of R2-ALP. Avoid foaming.

Stability of working reagent : 10 days at 2-8°C
3 days at 15-25°C

Protect from light and avoid contamination, Slightly yellow colour of working reagent is normal and does not influence the result.

CONCENTRATIONS IN THE TEST

2-amino-2-methyl-1-propanol (AMP)	350 mmol/l
Mg2+	2.0 mmol/l
Zn2+	1.0 mmol/l
HEDTA	2.0 mmol/l
p-nitrophenylphosphate	16.0 mmol/l

WARNINGS AND NOTES

- Product for in vitro diagnostic use only.
- The reagents contain 0.09% sodium azide as a preservative.
- Avoid contact with skin and mucous membranes.
- During the reaction p-nitrophenol is produced. Do not swallow or inhale, avoid contact with skin.
- The reagents are usable when the absorbance of the working
- Reagent is less than 1.250 (read against distilled water,
- Wavelength λ=405 nm, cuvette l=1 cm, at temp. 25°C).

ADDITIONAL EQUIPMENT

- Automatic analyzer or photometer able to read at 405 nm
- Thermostat at 30°C or 37°C
- General laboratory equipment

SPECIMEN

Serum, heparinized plasma free from hemolysis.
Do not use EDTA, citrate and oxalate as anticoagulants because of ALP activity inhibition ALP activity remains stable in specimen up to 4 hours at 15-25ºC but it is recommended to perform the assay with freshly collected samples. Freezing of sample causes a loss of enzyme activity. Frozen specimens should be thawed and kept at room temperature for 18 to 24 hours before measurement to achieve full enzyme reactivation.

PROCEDURE

These reagents may be used both for manual assay and in several automatic analyzers. Applications for them are available on request.

Wavelength	405 nm
Temperature	37°C
Cuvette	1 cm

Pipette into the cuvette:

Reagent	Test(T)
R1 ALP reagent	800 µl
R2 ALP reagent	200 µl
Bring to assay temperature, then add	
Sample	20 µl

Mix and incubate at adequate temperature. After about 60 secs read the absorbance against air or water. Repeat the reading after exactly 30 secs interval for next 3 readings. Calculate the mean absorbance change per minute ΔA/min.)

CALCULATION

ALP activity [U/L] = ΔA/min. x 2720 (factor)

REFERENCE VALUES

Female	20 - 50 years	42 - 98 U/L
	>60 years	53 - 141 U/L
Male	20 - 50 years	53 - 128 U/L
	>60 years	56 - 119 U/L

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL

To ensure adequate quality control, each run should include assayed normal and abnormal controls. If commercial controls are not available it is recommended that known value samples be aliquoted, frozen and used as controls.

PERFORMANCE CHARACTERISTICS

- **Sensitivity / Limit of Quantitation :** 8.8 U/L.
- **Linearity :** up to 1600 U/L.
- **Specificity / Interferences**
  Haemoglobin up to 3.75 g/dl, ascorbate up to 62 mg/l, bilirubin up to 20 mg/dl and triglycerides up to 500 mg/dl do not interfere with the test.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

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SYSTEM PARAMETERS

Method	Kinetic
Wavelength	405 nm
Zero Setting	Distilled water
Temperature Setting	37°C
Incubation Temperature	37°C
Incubation Time	----
Delay Time	60 secs
Read Time	90 secs
No.of.Reading	3
Interval time	30 secs
Sample Volume	0.02 ml (20 µl)
Reagent Volume	1.0 ml (1000 µl)
Standard Concentration	-----
Units	U/L
Factor	2720
Reaction slope	Increasing
Linearity	1600 U/L

IVD CE

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