ROBONIK®

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prietest™ *...Clinical Chemistry Reagents*

ALKALINE PHOSPHATASE

In vitro diagnostic test kit, for professional use only

INTENDED USE : Quantitative in vitro determination of activity of Alkaline Phosphatase in serum or plasma on photometric systems.

ORDERING INFORMATION	**Pack Size**	**Cat No.**
	1 X 25 ml	ALP 01 25
	2 X 50 ml	ALP 02 50
	4 X 50 ml	ALP 04 50
	2 X 500 ml	ALP 02 500

CLINICAL SIGNIFICANCE : Alkaline Phosphatase is an enzyme of the Hydrolase class of enzymes and acts in an alkaline medium. It is found in high activity on the liver, biliary tract epithelium and in the bones. Normal levels are age dependent and increase during bone development. Increased levels are associated mainly with liver and bone disease, hepatotoxicity caused by drugs or osteomalacia, as well as obstruction.

METHOD :
IFCC method, AMP Buffer, Kinetic, Colorimetric.

PRINCIPLE :
p-Nitrophenyl phosphate is converted to p-nitrophenol and phosphate by alkaline phosphatase. The rate of formation of p-Nitrophenol is measured as an increase in absorbance which is proportional to the ALP activity in the sample.

p-Nitrophenyl phosphate $\xrightarrow{\text{ALP}}$ p-Nitrophenol + Phosphate

REAGENTS :

COMPONENTS AND CONCENTRATIONS:

R1:	2 - Amino-2-Methyl-1-Propanol	0.35 mol/l
	Magnesium Sulphate	2.0 mmol/l
	Zinc Sulphate	1.0 mmol/l
R2 :	p-Nitrophenyl Phosphate	16.0 mmol/l
	Preservative & Stabilizer	

STORAGE INSTRUCTIONS AND REAGENT STABILITY:
The reagents are stable up to the end of the indicated date of expiry on the vial label, if stored at 2 to 8°C, protected from light and contamination is avoided. Do not freeze the reagent ! Discard the Reagent if found turbid or in case the absorbance of Working Reagent is greater than 0.8 AU at 405 nm against distilled water.

WARNINGS AND PRECAUTIONS :
Take the necessary precautions for the use of laboratory reagents.

WASTE MANAGEMENT :
Please refer to local regulation requirements.

REAGENT PREPARATION :
1) Two Reagent procedure : The reagents are ready-to-use.
2) Mono Reagent Procedure : Mix four volumes of reagent R1 with one volume of reagent R2. Stability of working reagent solution : Four Weeks at 2 to 8°C.

MATERIAL REQUIRED BUT NOT PROVIDED :
NaCl solution 9 g/l, General laboratory equipment, Analyser / Photometer, pipettes etc.

SPECIMEN :
Serum free from hemolysis, Heparinized or EDTA plasma.
Stability in serum / plasma
Sera are stable 7 days at 2 to 8°C.
Discard contaminated specimens.

ASSAY PROCEDURE 1: Two Reagent procedure
Application sheets for automated systems are available on request.
Wavelength : Hg 405 nm
Optical path : 1 cm
Temperature : 37°C
Mode : Kinetic
Bring all the contents of the kit to Room Temperature prior to use.
Read rate of change of absorbance of sample against distilled water or air.
Label the test tube as sample, control and pipette into respective test tube the reagent, sample, control sample as per the table given below :

	Sample / Control
Reagent R1	800 µl
Reagent R2	200 µl

Mix and Incubate at 37°C for 2 minutes then add

Sample / Control	20 µl

Mix and after a 60 seconds incubation at 37°C measure the change of absorbance per minute (ΔA/minute) during 180 seconds.

ASSAY PROCEDURE 2 : Mono Reagent procedure
Label the test tube as sample, control and pipette into respective test tube the reagent, sample, control sample as per the table given below :
Prewarm working reagent at 37°C for two minutes prior to addition of sample.

	Sample / Control
Working Reagent	1000 µl
Sample / Control	20 µl

Mix and after a 60 seconds incubation at 37°C measure the change of absorbance per minute (ΔA/minute) during 180 seconds.

CALCULATION :
At 405 nm with mono reagent procedure and two reagent procedure for 1 cm path light cuvette
Activity of Sample (U/L) = (Δ A/Min) X 2712

TEMPERATURE CONVERSION FACTORS :
To correct result to other temperatures multiply by factor shown in table

Assay Temperature	**Conversion Factor to**		
	25°C	30°C	37°C
25°C	1.00	1.22	1.64
30°C	0.82	1.00	1.33
37°C	0.61	0.75	1.00

CALIBRATION :
For the calibration of automated photometric systems use of the commercially available calibrator is recommended.

QUALITY CONTROL :
To ensure adequate quality, use of the commercially available control sera is recommended.

PERFORMANCE CHARACTERISTICS :

MEASURING RANGE :
The test has been developed to determine ALP activity within a measuring range from 5 to 700 U/L. When values exceed higher limit of the range, such samples should be diluted 1 + 1 with NaCl solution (9 g/l) and the result multiplied by 2.

SPECIFICITY / INTERFERENCE :
No interference was observed by Ascorbic Acid up to 30 mg/dl, Bilirubin up to 15 mg/dl, Glucose up to 500 mg/dl and lipemia up to 600 mg/dl Triglycerides. A list of drugs and other interfering substances with ALP determination has been reported by Young et al.

SENSITIVITY / LIMIT OF DETECTION :
The lower limit of detection is 5 U/L.

PRECISION :

Intra-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	417.2	3.79	0.91
Sample 2	234.1	2.13	0.91
Sample 3	260.8	2.54	0.97

Inter-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	81.34	1.57	1.94
Sample 2	98.30	1.23	1.25
Sample 3	106.4	0.95	0.89

METHOD COMPARISON :
A comparison between Robonik Prietest ALP (y) and a commercially available test (x) using 20 samples gave following results:
Linear Regression : y = 0.6009 x - 10.896 U/L
Correlation Coefficient : r = 0.9748

REFERENCE RANGE :
Men : 44 to 132 U/L Women : 44 to 147 U/L Children : 50 to 350 U/L
It is recommended that each laboratory should assign its own reference range.

LITERATURE :

1. Tietz, N.W., Clinical guide to laboratory tests. 3rd Ed., (W.B. Saunders eds. Philadelphia USA), (1995), 46.
2. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed. AACC Press, 1995.

INSTRUMENT APPLICATION
***prietest* TOUCH**

Name : ALP , Mod : KIN
Pri.: 405 , Sec.: 0
Temp: 37C , KF : 1.000
Vol : 500ul , Unit : U/L
Lag : 60 , Read : 180
Blk : N, QC : Y, Norm : Y
Std : N , Factor : 2712
Normal HI = 147
Normal LO = 44
QCNH : *
QCNL : *
QCABH = *
QCABL = *
Init. OD : = 0.8 H
Max Delta/Min : = 0.258
Rgnt. Linearity : 700
NOTE :
* Indicates user definable parameter.
NA Implies Not Applicable

PARAMETERS FOR INSTRUMENT SETTING	
TEST NAME	ALP
Reaction	Kinetic
Reaction Slope	Increasing
Wavelength 1	405 nm
Temperature	37°C
Zero Setting	Distilled Water/Air
Factor	2712
Units	U/L
Sample Volume	20 µl
Reagent Volume	1000 µl
Lag Time	60 Seconds
Read Time	180 Seconds
Reference Range	44 to 147
Reagent Linearity	700
Max Delta/Min	0.258
Initial OD	< 0.8

ROBONIK®

An ISO 9001 : 2008 Certified Company
An ISO 13485 : 2012 Certified Company

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