Spectrum For Diagnostic Industries

Alkaline phosphatase (ALP) Kinetic - IFCC(1 + 1)

IVD

REF.	Pack size
159 04 025	(4 x 25 ml) 100 tests

Intended Use

Alkaline Phosphatase reagent is intended for the in-vitro quantitative and diagnostic determination of ALP in human serum on both automated and manual systems .

Introduction

Alkaline phosphatase (ALP) catalyzes the hydrolysis of a wide variety of physiologic and non-physiologic phosphoric acid esters in alkaline medium (pH optimum 10). The liver and biliary tract are the source of alkaline phosphatase in normal sera. Normal alkaline phosphatase levels are age dependent being higher in children and adolescents in comparison to adults. ALP is one of the tests of choice for evaluating cholestasis and obstructive juandice. Elevated levels are found in many diseases including hepatitis, cirrhosis, malignancy, and in bone diseases.

Method

Kinetic method according to the International Federation of Clinical Chemistry (IFCC)

Principle

Alkaline phosphatase (ALP) hydrolyzes *p*-Nitrophenylphosphate (*p*-NPP) to *p*-Nitrophenol and phosphate.

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

The increase of absorbance per minute at 405 nm is proportional to the enzyme activity.

Reagents

Buffer Reagent

2-Amino-2-Methyl-1-Propanol (pH 10.3)	2.0 mol/L
$MgCl_2$	2.0 mmol/L

Substrate

p-Nitrophenylphosphate	16 mmol/L

Reagents preparation, storage and stability

All reagents are stable till expiration date stated on label when stored refrigerated at 2 - 8 ^{o}C.Once opened, the reagent is stable for 2 months at the specified temperature.
Prepare working solution by adding equal volumes from R1 and R2. Working solution is stable for 4 weeks at 2 – 8 ^{o}C or 5 days at 15 - 25 ^{o}C.

Deterioration

Do not use liquizyme ALP reagent if it is turbid or if the absorbance of the working reagent is more than 2.2 at 405 nm. Failure to recover control values within the assigned range may be an indication of reagent deterioration.

Precautions and Warnings

Do not ingest or inhalate. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

Specimen collection and preservation

Non-haemolyzed fresh serum is the preferred specimen. Heparin is the only acceptable anticoagulant. Alkaline phosphatase activity may slowly increase in serum samples stored at room temperature. Previously frozen or lypholized sera may show a marked decrease in values immediately.The activity then increases to the initial values, and the rate of this increase is time and temperature dependent.

Stability: 2 months at -20 ^{o}C ; 4 weeks at 4 – 8 ^{o}C; 7 days at 20 – 25 ^{o}C

Procedure

Wavelength	405 nm (400 – 420 nm)
Optical path	1 cm
Assay type	Kinetic
Direction	Increase
Sample : Reagent Ratio	1 : 100
Temperature	37 ^{o}C
Equilibration time	1 minute
Read time	1 to 3 minutes
Zero adjustment	Against air
Reagent Blank Limits	Low 0.2 AU High 2.2 AU
Sensitivity	5 U/L
Linearity	750 U/L

Working solution 1.0 ml (or add 0.5 ml R1+ 0.5ml R2)

Specimen 10 µl

Mix, read initial absorbance after 1 minute and start timer simultaneously. Read again after 1, 2 and 3 minutes.
Determine the mean absorbance change per minute (ΔA/min).

Calculation

To calculate the alkaline phosphatase (ALP) activity,use the following formula: U/L = 5454 × ΔA 405 nm/min

Quality control

Normal and abnormal control serum of known concentration should be analyzed with each run.

Sensitivity

When run as recommended, the minimum detection limit of this assay is 5.0 U/L.

Linearity

The reaction is linear up to alkaline Phosphatase concentration of 750 U/L; specimens showing higher concentration should be diluted 1+5 with physiological saline and repeat the assay (result×6).

Interference

Haemolysis
A 200 mg/dL haemoglobin results in a 10% negative bias.

Icterus
No interference up to bilirubin level of 40 mg/dL.

Lipemia
No interference up to 1000 mg/dL.

Expected Values

1- Males
a) 20 - 50 years 53-128 U/L
b) > 60 years 56-119 U/L
2- Females
a) 20 - 50 years 42-98 U/L
b) > 60 years 53-141 U/L
3- Childern: 1 - 12 years <460 U/L

Temperature conversion factor is 1.22 (25 ⟶ 30 °C) and 1.52 (25 ⟶ 37 °C).

Performance characteristics

A comparison between ALP reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.990 was obtained.

Precision
Within run (Repeatability)

	Level 1	Level 2
n	20	20
Mean (U/L)	177.7	359.7
SD	1.71	1.5
CV%	0.96	0.43

Run to run (Reproducibility)

	Level 1	Level 2
n	20	20
Mean (U/L)	178.5	365.5
SD	1.82	1.86
CV%	1.15	0.55

Waste Disposal

This product is made to be used in professional laboratories.
Please consult local regulations for a correct waste disposal.
S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental contamination.
S61: avoid release in environment. refer to special instructions/safety data sheets.

References

1.Moss DW, Henderson AR, Kachmar JF.
2.Tietz NW, Rinker AD, Shaw LM. IFCC methods for the measurement of catalytic concentration of enzymes. IFCC method for alkaline phosphatase . J Clin Chem Clin Biochem.
3.Zawta B, Klein G, Bablok W. Temperaturumrechnung in der Klinischen Enzymologie? Klin lab.

SYMBOLS IN PRODUCT LABELLING

IVD For in-vitro diagnostic use
LOT Batch Code/Lot number
REF Catalogue Number
Consult instructions for use
Temperature Limitation
Use by/Expiration Date
CAUTION. Consult instructions for use
Manufactured by

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