LIQUIZYME

ALKALINE PHOSPHATASE SINGLE REAGENT

(AMP Optimized IFCC Method)

BEACON

Code	Product Name	Pack Size
LS002A	Liquizyme Alkaline Phospatase Single Reagent	25 T

Intended Use

Diagnostic reagent for quantitative *in vitro* determination of ALP in human serum or plasma.

Clinical Significance

Human ALP consists of a group of enzymes which hydrolyse phosphates at an alkaline pH. ALP is found in practically all tissues of the body but in high concentrations in the osteoblasts of bone, liver, placenta, kidney, intestinal wall and lactating mammary glands. In adults the ALP normally found circulating in the serum is largely derived from the liver. In children or in adolescents going through pubertal growth spurts, these is an additional contribution from bone and this accounts for the higher reference interval for these groups. Pregnancy also raises the normal values of ALP. Raised ALP levels are often observed in bone disease or liver disease involving the biliary tract. If the source of the isoenzyme is not apparent then estimation of GGT may help differentiate between the two. A raised GGT in the presence of a raised ALP would suggest the liver is the primary source. Increased ALP (usually normal GGT) is seen in Osteomalacia and Rickets, primary hyperparathyroidism with bone involvement, Pagets disease, secondary carcinoma in bone and some cases of osteogenic sarcoma. Increased levels of ALP (usually with a raised GGT) is seen in cholestasis, hepatitis, cirrhosis, space occupying lesions and malignancy with bone or liver involvement or direct production. Low levels of ALP may be observed in conditions which cause arrested bone growth or in hypophosphatasia.

Principle

The method according to IFCC recommendation. This method utilises 4-nitrophenyl phosphate as the substrate. Under optimised conditions ALP present in the sample catalyses the following reaction.

$$\text{AMP} + \text{4-NNP} + H_2O \xrightarrow[Mg_{2+} \,/\, \text{Alkaline pH}]{\text{ALP}} \text{4-nitrophenol} + \text{phosphate}$$

At the pH of the reaction, 4-nitrophenol has anintense yellow colour. The reagent also contains a metal ion buffer system to ensure that optimal concentrations of Zinc and Magnesium are maintained. The metal ion buffer can also chelate other porentially inhibitory ions which may be present. The reaction is monitored by measuring the rate of increase in absorbance at 405 or 415 nm which is proportional to the activity of ALP in the serum.

Reagent Composition

Reagent 1 : Alkaline Phosphatase Reagent

2-AMP : >250 mmol/L
Mg+2 : >2 mmol/L
Zn+2 : >10 mmol/L
HEDTA : >1.5 mmol
PNPP : < 10 mmol/l

Reagent Preparation

Reagent is liquid, ready to use.

Stability And Storage

The unopened reagents are stable till the expiry date stated on the bottle and kit label when stored at 2–8°C.

Material Required But Not Provided

- Clean & Dry container.
- Laboratory Glass Pippetes or Micropippetes & Tips.
- Colorimeter or Bio-Chemistry Analyzer.

Specimen Collection And Handling

Use serum, plasma (Heparin). It is recommended to follow NCCLS procedures (or similar standardized conditions).

Stability In Serum / Plasma :

4 hours : at 20 – 25°C
3 days : at 4 – 8°C
2 months : at -20°C

Discard contaminated specimens.

Quality Control

It's recommended to run normal and abnormal control sera to validate reagent performance.

Unit Conversion

U/l x 0.017 = µkat/l

Expected Values

Children (3-15 yrs) : 104 - 309 U/L
Adults : 25 - 140 U/L

It is recommended that each laboratory verify this range or derives reference interval for the population it serves.

Performance Data

Data contained within this section is representative of performance on Beacon system. Data obtained in your laboratory may differ from these values.

Limit of quantification : 10.0 U/L
Linearity : 1600 U/L
Measuring range : 10.0 – 1600 U/L

Intra-assay precision Within run (n=20)	Mean (U/L)	SD (U/L)	CV (%)
Sample 1	83	1.60	1.92
Sample 2	399	5.24	1.31

Inter-assay precision Run to run (n=20)	Mean (U/L)	SD (U/L)	CV (%)
Sample 1	95	0.65	0.69

Comparison

A comparison between Beacon ALP (y) and a commercially available test (x) using 20 samples gave following results :

y = 1.006 x - 1.19 U/L

r = 0.998

Interferences

Following substances do not interfere:

haemoglobin up to 5 g/l, bilirubin up to 40 mg/dl, triglycerides up to 2000 mg/dl.

Waste Management

Please refer to local legal requirements.

Assay Procedure

Wavelength : 405 nm

Cuvette : 1 cm

Addition Sequence	Volume
Reagent 1	1000 µl
Sample	20 µl

Mix well and read the initial absorbance after 1 minutes and repeat the absorbance reading after every 1, 2, 3 minute. Calculate 1 minute absorbance change (ΔA/min).

Calculation

Using factor:

ALP (U/L) = f x ΔA/min

f = factor

f = 2764 (at 405 nm)

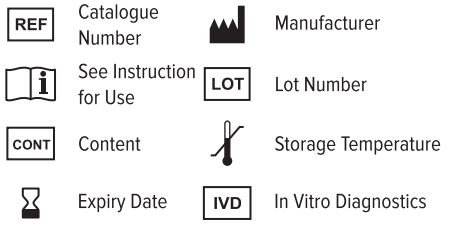
Assay Parameters For Photometers

Mode	Kinetic
Wavelength 1 (nm)	405
Sample Volume (µl)	20
Reagent Volume (µl)	1000
Lag time (sec.)	60
Kinetic Interval (sec.).	60
No. of Interval	3
Kinetic Factor	2764
Reaction temp. (°C)	37
Normal Low (U/L)	25
Normal High (U/L)	140
Linearity Low (U/L)	10
Linearity High (U/L)	1600
Blank with	Water
Unit	U/L

References

1. Zilva JF, Panall PR, "Plasma Enzymes in Diagnosis" in Clinical Chemistry in Diagnosis and Treatment. Lloyd London 1979: Chapter 15 : 343.
2. IFCC method for the measurement of ALP J. Clin. Chem. Clin. Biochem. 1983: 21: 731-48.
3. Young DS. Effects of Drugs on Clinical Laboratory Tests. Third Edition 1990 : 3 : 19.25.
4. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. Burtis, C.A., Ashwood, E.R., Bruns, D.E.; 5th edition, WB Saunders Comp., 2012.
5. Kaplan and Pesce (Eds.) Clinical Chemistry, Theory analysis and correlation. Second Edition. CV Mosby Co. 1989.

Symbols Used On Labels



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