LiquiMAX Alkaline Phosphatase

(DGKC Method / DEA Buffer)

ORDERING INFORMATION

Ref. No.	Pack Size	Presentation
AVALP2 - 50	(R1 5 x 8ml + R2 1 x 10ml)	Two Liquid Reagents
AVALP2 - 100	(R1 4 x 20ml + R2 2 x 10ml)	
AVALP2 - 200	(R1 8 x 20ml + R2 4 x 10ml)	

INTENDED USE:

LiquiMAX Alkaline Phosphatase Kit is use for the quantitative determination of alkaline Phosphatase (ALP) in human serum.

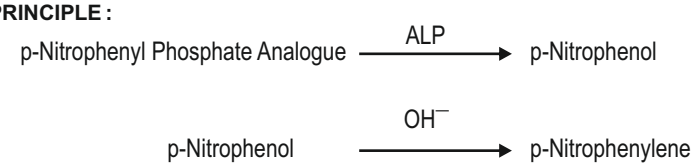
PRODUCT FEATURES:

1. Liquid Stable, Ready to use Two Reagents (4 parts R1+1 part R2).
2. 2 minutes increasing kinetic reaction (10 Sec Delay+120 Sec Measuring).
3. PNPP Analogues are used.
4. Measuring Wavelength 405 nm.
5. Kinetic factor 3104 at 37° C.
6. Linearity 1500 IU/L.
7. Serum is the only specimen.
8. Available as multipurpose reagents and dedicated system packs.

CLINICAL SIGNIFICANCE

Alkaline phosphatase in serum consists of four structural genotypes: the liver-bone-kidney type, the intestinal type, the placental type and the variant from germ cells. It occurs in osteoblasts, hepatocytes, kidneys, spleen, placenta, prostate, leukocytes and the small intestine. The liverbone-kidney type is particularly important. A rise in the Alkaline Phosphatase activity occurs with all forms of cholestasis, particularly with obstructive jaundice. It is also elevated in diseases of the skeletal system, such as Paget`s disease, hyperparathyroidism, rickets and osteomalacia, as well as with fractures and malignant tumors. A considerable rise in the Alkaline Phosphatase activity is sometimes seen in children and juveniles. It is caused by increased osteoblast activity following accelerated bone growth. Various reference values for the purposes of clinical evaluation have been assigned to differing age groups. In 1946, Bessey, Lowry and Brock published a method for the determination of Alkaline Phosphatase using p-nitrophenyl phosphate as substrate buffered with glycine/NaOH. in 1967, Hausamen et al improved upon the method by using Diethanolamine as buffer. The "optimized standard method" assay described here meets the 1972 recommendations of the "Deutschen Gesellschaft für Klinische Chemie (German Society of Clinical Chemistry)".

PRINCIPLE :



Serum ALP hydrolyses p-nitrophenyl phosphate to p-nitrophenol (colorless). Deprotonation of the phenolic group gives rise to the yellow colored p-nitrophenylene anion and the concentration of this anion is determined by measuring the absorbance at 405nm.

STORAGE AND STABILITY

When reagents are stored properly at 2-8°C and the contamination is avoided, they are stable up to the expiry date mentioned on the labels and kit box.

KIT COMPONENTS

1. Buffer Reagent R1
2. Substrate Reagent R2

COMPOSITION:

R1	:	Diethanolamine	pH 9.7	1.5 Mol/l
	:	Magnesium chloride		0.6 mMol/l
R2	:	p-Nitophenylphosphate Analogue		0.070 mMol/l
		Sodium Azide		0.10 %

REAGENT RECONSTITUTION & STABILITY

Reagent are liquid stable no need for reconstitution.

When the reagent is stored properly at 2-8°C & the contamination avoided, it is stable up to the expiry date mention on the label & kit box.

MATERIAL REQUIRED BUT NOT PROVIDED

Laboratory Instrumentation, Spectrophotometer UV/VIS with thermostatic cuvette holder or clinical chemistry analyzer: semi auto, calibrated micropipettes, glass or high quality polystyrene cuvettes, test tube/rack, heating bath controls, saline.

REAGENT DETERIORATION

Discard any turbid reagent if reagent absorbance exceeds 2.0 at 405 nm against distilled water.

WARNINGS AND PRECAUTIONS:

- Reagent may contain some non reactive and preservative components. It is recommended to handle carefully, avoiding contact with skin and ingestion.
- Specimen should be considered infectious and handled appropriately.
- Contamination by soap or glycerol will affect this assay.
- Perform the test according to the general " Good Laboratory Practice" GLP guidelines.

SPECIMEN COLLECTION & STORAGE

UNHEMOLYSED SERUM Is the preferred specimen.

Samples are stable for a week at 2-8°C and for a month at -10°C. Samples should be brought to room temperature prior to use.

SYSTEM PARAMETERS

Reaction type (mode)	:	kinetic
Reaction direction	:	increasing
Wave length	:	405nm
Flow cell temp.	:	37°c
Zero setting with	:	distilled water
Delay time	:	10 seconds
Measuring time	:	120 seconds
reagent volume	:	1 ml (R1 0.8 ml + R2 0.2 ml)
Sample volume	:	25 µl
Factor	:	3104
Linearity	:	1500
Units	:	IU/L
Low normal	:	80 (adults)
High normal	:	300 (adults)

TEST PROCEDURE

Reagent	Sample/Control
R1	0.8 ml
R2	0.2 ml
Sample/Control	25 ml

Mix well and after 10 Sec incubation (Delay) and measure the change of optical density during the next 120 seconds (Measuring Time) against distilled water at 405 nm as follows.

Ao Exactly after 10 Seconds.
A1, A2 Exactly after 60 seconds for 120 Seconds

CALCULATION

Calculate the average change in absorbance per minute (△ A/min) and multiply by the corresponding factor.

ALP activity [IU/L]: △A/min x 3104

EXPECTED VALUES

		25°C	30°C	37°C
Children				
1-15years	IU/L	<480	<596	<810
Adults	IU/L	<170	<220	<300

QUALITY CONTROL & CALIBRATION

It is recommend to perform internal quality control with assayed normal (BioNorm) and assayed abnormal (BioPath), to confirm the validity of the test and assure the accuracy of patient result.

Using the recommended calibrator (Avecon) or the standard included, calibrate the assay:

a) When using a new reagent or lot.

b) When QC values are out of range.

PERFORMANCE CHARACTERISTICS

1. Linearity
Linearity : 1500 IU/L

2. Sensitivity/ Limit Of Detection (LOD)
The lower limit of detection is 2 IU/L

3. Interferences
No interference is absorbed by triglycerides up to 2200mg/dl, bilirubIn up to 42mg/dl, hemoglobin up to 150mg/dl, intra lipids up to 1200 mg/dl and ascorbic acid upto 32mg/dl

4. Precision

Intra-assay precision	mean	SD	CV
N=20	(IU/L)	(IU/L)	(%)
Sample 1	143.1	2.30	1.59
Sample 2	448.4	6.60	1.46
Sample 3	449.2	5.90	1.32

Inter-assay precision	mean	SD	CV
N=20	(IU/L)	(IU/L)	(%)
sample 1	393.8	6.20	1.80
sample 2	892	8.00	1.00
sample 3	1400	50.00	3.40

5. Method Comparison
A comparison of the LiquiMAX ALP (y) with a commercial obtainable assay (x) gave following results with 80 samples.
y = 0.992 x + 2.64; r = 0.991

LIMITATION

The test has been developed to determine Alkaline Phosphatase activities which correspond to maximal A/min of 0.483.

If such value is exceeded the sample should be diluted 1+9 with NaCl solution (9g/l) and the results be multiplied by 10.

WASTE DISPOSAL

Reagents must be disposed off in accordance with local regulations.

Notes :

1. Avoid contact with skin and mucous membranes. The reagents contain sodium azide as preservative. Do not swallow.
2. During the reaction p-nitrophenol is produced. This substance is poisonous when inhaled, swallowed or when absorbed through the skin. If the reaction mixture comes into contact with skin or membranes, wash copiously with water and consult a doctor.

REFERENCES

1. Bablok W et al. A General Regression Procedure for Method Transformation. J Clin Chem Clin Biochem 1988;26:783-790.
2. Bessey OAH et al. J Biol Chem 1946;164:321
3. Empfehlungen der Deutschen Gesellschaft für Klinische Chemie. Standard-Methode zur Bestimmung der Aktivität der alkalischen Phosphatase. Z klin Chem u klin Biochem 1972;10:191.
4. Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-474.

Symbols Used on Pack

REF	Catalogue Number	⚠	Warning/Caution
LOT	Batch No.	IVD	In vitro diagnostic device
	Manufacturing Date		Storage Limit
	Expiry Date		Consult instruction for use
	Manufacturer		Keep away from sunlight
	Keep Dry		Do not use if package is damaged
Σ	Contains sufficient no. of test		

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