ANA BIO ISP ALKALINE PHOSPHATASE

(p-NPP with DEA buffer method)

For Miura Instruments

Intended Use
Alkaline phosphatase is a reagent kit used for the quantitative determination of Alkaline phosphatase activity in human serum or plasma based on kinetic method using p-nitrophenyl phosphate (p-NPP).

Principle
Alkaline phosphatase cleaves p-nitrophenyl phosphate (p-NPP) into p-nitrophenol and phosphate. p-nitrophenol is a yellow colour compound in alkaline medium. The intensity of the yellow colour formed is directly proportional to the Alkaline phosphatase activity in specimen.

p - nitrophenyl phosphate + H_2O $\xrightarrow{ALP}$ Phosphate + p - nitrophenol

Components & Concentration of Reagents

Reagent	Component	Concentration
Reagent 1	DEA buffer, pH 9.8	1 mol/L
	Magnesium chloride	≥ 0.5 mmol/L
	Stabilizers, excipients & surface active agents	
Reagent 2	p-NPP	≥ 10 mmol/L
	Stabilizers, excipients & surface active agents	

Reagent storage and stability
The kit should be stored at 2° - 8℃ and is stable till the expiry date indicated on the label. **DO NOT FREEZE THE REAGENT.** Contamination of the reagent should be strictly avoided.

Reagent Preparation
Liquid reagents ready for use. After opening the reagents of R1 and R2 are stable for 30 days if closed, stored at 2° - 8℃, and protect from direct light and contamination. Do not mix different batches.

Specimen collection and preservation
Blood should be collected in a clean and dry container. Haemolysed specimen should be avoided as it may falsely elevate results. EDTA, citrate and oxalate inhibit Alkaline Phosphatase activity and should not be used as anticoagulant.
For plasma separation any of the following two anticoagulants may be used:

- HEPARIN 200 IU/ml of blood
- SODIUM FLUORIDE 10 mg/ml of blood

Serum/plasma should be separated as quickly as possible from cells. Alkaline Phosphatase is stable for 4 days at 2° - 8℃ and several months when stored at -20℃.

Automation
This kit, though developed and manufactured to be used as manual assay and with I.S.E. Miura Analyzer, can be used also with other analyzers able to meet the specifications indicated in section "Reaction conditions – Test procedure" Application sheets are available for automatic instruments.
All applications not explicitly approved by KDPL. Cannot be guaranteed in terms of performance, and must there be established by the operator.

Calibration
For Calibration use the "Multicalibrator"

Calibration Stability
For the instrumentation series Miura, the calibration is recommended to be done every 10 days.

Materials required but not supplied in the kit
Calibrators and controls

Assay guidelines for Analyzer I.S.E. Miura

Analyte Name	Alkaline Phosphatase DEA
Method Code	ALP-DEA
Type	Kinetic Substrate Start
Unit	IU/L
Filter F1	405 nm
Blank in	Used

Step	Reaction Volume	U.M.
Volume reagent R1	200	µl
Sample Volume	5	µl
Incubation R1,S → R2	60	Sec.
Volume reagent R2	50	µl
Final Incubation	60	Sec.
Kinetic reading time	192	Sec.

Normal range
Serum/Plasma

	Unit	25℃	30℃	37℃
Adult (≥ 15 years)	IU/L	60 – 170	78 – 221	108 - 306
Child (< 15 years)	IU/L	150 – 450	195 – 585	210 - 810

Note: Expected range varies from population to population and each laboratory should establish its own normal range.

Limitation
The reagent is linear up to 1000 IU/L. For higher value, dilute sample with normal saline and perform the assay. Multiply the final result by dilution factor to get the real value.

Quality Control
To ensure adequate quality control, it is recommended that each batch should include a normal and an abnormal commercial reference control serum. It should be realized that the use of quality control material checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware, Wavelength setting, Expiration date of reagents and accuracy of prob aspiration.

Accuracy-Recovery
ALP added to a serum matrix containing known amounts of ALP gave an average recovery of 99%.

Interference
There is no significant interference in samples containing upto 40 mg/dl of bilirubin, 400 mg/dl of haemoglobin, 30 mg/dl of ascorbic acid and up to 2000 mg/dl of Triglycerides.

Precision of the Method

Within-run					
Range	U.M	Mean	S.D.	C.V.(%)	No. run
Low	IU/L	176.55	3.65	2.07	20
High	IU/L	532.16	3.81	0.72	20
Between run					
Range	U.M	Mean	S.D.	C.V.(%)	No. run
Low	IU/L	175.06	4.07	2.32	60
High	IU/L	532.49	3.59	0.67	60

Sensitivity
At 405 nm the activity of ALP can estimate up to 3 IU/L.

References

1. Hendry, R. J., "ENZYMES" in Clinical Chemistry Principle and techniques, Harper & Row Publishers, New Yark, 815 (1974).
2. Young, D.S. et al, Clin. Chem. 18, 1041, (1972).

Symbols

IVD	In Vitro Diagnostics		Caution
LOT	Batch No.		Product Expiry Date
CONT	Content		Manufactured By
	Read Instructions		Date of Manufacture
	Storage Temperature		Keep Dry
REF	Catalogue No.		Fragile
			Keep away from sun light

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