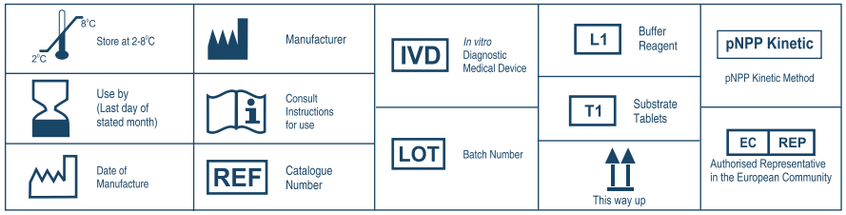
SBio ALKALINE PHOSPHATASE KIT (DEA)

(pNPP Kinetic Method)

(For invitro diagnostic use only)

REF	90190515
Pack Size	5 x 15 ml

CE



INTENDED USE

Alkaline Phosphatase Kit is used for the determination of Alkaline Phosphatase Activity in serum.

SUMMARY

Alkaline Phosphatase (ALP) is an enzyme of the Hydrolase class of enzymes and acts in an alkaline medium. It is found in high concentrations in 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. Moderate increases are seen in Hodgkins disease and congestive heart failure.

PRINCIPLE

ALP at an alkaline pH hydrolyses p-Nitrophenylphosphate to form p-Nitrophenol and Phosphate. The rate of formation of p-Nitrophenol is measured as an increase in absorbance which is proportional to the ALP activity in the sample.

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

EXPECTED VALUES

Serum (Adults) : 80-290 U/L at 37°C
(Children) : 245-770 U/L at 37°C

It is recommended that each laboratory establish its own normal range representing its patient population*.

CONTENTS

	5 x 15 ml
L1 : Buffer Reagent	80 ml
T1 : Substrate Tablets	5 Nos.

COMPOSITION

DEA Buffer 1M pH 10.3; Magnesium Chloride 0.5 mM; pNPP 10 mM.

STORAGE / STABILITY

Contents are stable at 2-8°C till the expiry mentioned on the labels.

REAGENT PREPARATION

Working Reagent: Dissolve 1 Substrate Tablet in 3.2 ml (10 x 3 ml pack) or 15 ml (5 x 15 ml pack) of Buffer Reagent.
This working reagent is stable for atleast 15 days when stored at 2-8°C.
The substrate is light & temperature sensitive. Take adequate care, especially after reconstitution.

SAMPLE MATERIAL

Serum. Free from hemolysis, ALP is reported to be stable in serum for 3 days at 2-8°C.

SAMPLE WASTE AND DISPOSAL

Do not reuse the reagent containers, bottles, caps or plugs due to the risks of contamination and the potential to compromise reagent performance.

This product requires the handling of human specimen. It is recommended that all human sourced material are considered potentially hazardous and are handled in accordance with the OSHA standard on blood borne pathogens.
Appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
Handle specimen, solid and liquid waste and test components in accordance with local regulations and NCCLS guidelines M29, or other published biohazard safety guidelines.

PROCEDURE

Wavelength/filter : 405 nm
Temperature : 37° C / 30° C / 25° C
Light path : 1 cm

MATERIALS REQUIRED BUT NOT PROVIDED

Photometer analyzer with standard thermostatic cuvette holder, micropipette and appropriate laboratory equipment.

Pipette into a clean dry test tube labeled as Test (T):

Addition Sequence	(T ml)
Working Reagent	1.0
Incubate at the assay temperature for 1 minute and add	
Sample	0.02

Mix well and read the initial absorbance A_0 after 30 Secs. & repeat the absorbance reading after every 1 & 2 minutes. Calculate the mean absorbance change per minute (ΔA/ min).

CALCULATIONS

ALP activity in U/L = ΔA/ min x 2754

QUALITY CONTROL

The following process is recommended for QC during the assay of Alkaline Phosphatase. *Define and establish acceptable range for your laboratory.

1. Two levels of control (Normal and Abnormal) are to be run on a daily basis.
2. If QC results fall outside acceptance criteria, recalibration may be necessary.
3. Review QC results and run acceptance criteria following a change of reagent lot.

SPECIFIC PERFORMANCE CHARACTERISTICS

Linearity:
The procedure is linear upto 700 U/L at 37°C. If the absorbance change (ΔA/ min) exceeds 0.250, use only the value of the first two minutes to calculate the result, or dilute the sample 1 + 9 with normal saline (NaCl 0.9%) and repeat the assay (Results x 10).

Limit of detection:
The limit of detection for Alkaline Phosphatase is 3 U/L.

Interferences:
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Precision:
Precision studies were performed with two controls using NCCLS protocol EP5-A. The results of the precision studies are shown below:

Sample	Within-run		Between-run		Total	
	Mean	CV%	Mean	CV%	Mean	CV%
Control 1	170.89	2.51	163.50	2.41	334.39	4.92
Control 2	409.47	2.13	423.11	2.04	832.58	4.17

Method comparison:
Comparative studies were done to compare our reagent with another commercial Alkaline Phosphatase Assay. No significant differences were observed. Details of the comparative studies are available on request.

TEMPERATURE CONVERSION FACTORS

Assay Temperature	Desired Reporting Temperature		
	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

NOTE

Samples having a very high activity show a very high initial absorbance. If this is suspected then dilute the sample and repeat the assay. The reagent may be used in several automated analyzers. Instructions are available on request. Do not use turbid, deteriorated or leaking reagents.

REFERENCES

1. Bowers, G. N. McCommb, R.B. (1972) Clin. Chem. 18:97
2. Recommendations of German Society for Clinical Chemistry, (1972)
3. Z. Clin. Chem. Bio. 10: 182.

Mfd. for:

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EC REP

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