ESTROM Alkaline Phosphatase (Lypho)
(pNPP Method)

ANGSTROM BIOTECH PVT. LTD

This reagent kit is for quantitative estimation of Alkaline phosphatase activity in serum or plasma.

PRINCIPLE :
The substrate p-nitrophenyl phosphate (pNPP) is hydrolysed by alkaline phosphatase present in the sample to p-nitrophenol (pNP) and phosphate in alkaline medium in presence of magnesium ions. pNP gives yellow colour. The intensity of colour corresponds to alkaline phosphatase activity and is read photometrically at 405 nm.

p-Nitrophenol Phosphate + H_20 → (Alkaline Phosphatase) → Phosphate + p-Nitrophenol

CLINICAL SIGNIFICANCE:
A physiological increase in alkaline phosphatase level due to active bone growth in children or in pregnancy from placental secretion is seen as a normal elevation. Increase in activity is seen in disease of bone, biliary tract and liver, certain drugs like androgens, anabolic steroids, estrogens, sulphonamides phenothiazine, tricyclic antidepressants, MAO inhibitors, antibiotics, leads to increase in alkaline phosphatase activity. Decreased activity seen in severe anemia, scurvy kwashiorkar and cretinism.

SPECIMEN COLLECTION AND STORAGE:
Fresh, clear and fasting unhemolysed serum is preferred.
Anticoagulants like oxalates, citrates and EDTA should be avoided.

PRECAUTION:
Estrom Alkaline Phosphatase reagent is for In Vitro diagnostic use only.

REAGENTS:
All the reagents are to be stored at 2-8° C.

	No. of bottles			
	50x2.2	10x5	25x1.1	
Reagent 1 (pNPP Substrate)	50	10	25	
Reagent 2 (Buffer)		2		10
1				

REAGENT RECONSTITUTION:
50x2.2 ml ,10 x 5 ml, 25x1.1 ml l: Reagent 1 (Substrate) is to be dissolved in Reagent 2 (Buffer).
Mix thoroughly. Keep for 5 minutes before use. Reconstituted reagent may be stored at 2-8° C, protected from light when not in use.

REAGENT STORAGE AND STABILITY:
All the reagents are stable up to expiry date stated on the label. Buffered substrate when stored at 2-8° C in a dark coloured bottle is stable for 7 days. Working reagent should be discarded if the blank exceeds 0.85 at 405 nm.

GENERAL INSTRUMENT PARAMETERS:

Reaction Type	: Kinetic
Slope of Reaction	: Increasing
Wavelength	: 405 nm
Flowcell Temperature	: 37° C
Reagent Volume	: 1.0 ml
Sample Volume	: 20 µl (0.02 ml)
Delay Time	: 30 seconds
Interval	: 30 seconds
No. of readings	: 3
Factor	: 2713
Units	: IU/L
Zero Setting	: Distilled water
Path length	: 1.0 cm

PROCEDURE:
Allow the sample and reagent to attain room temperature prior to use.

Dispense into test tubes	Test
Working Reagent	1.0 ml
Sample	20 µl

Mix and aspirate. Read absorbance at an interval of 30 seconds i.e. at 30, 60 and 90 seconds at 405 nm. Obtain the mean change in absorbance per minute (ΔA/min.)

LINEARITY:
This method is linear for Alkaline Phosphatase activity up to 1000 IU/L. For sample values exceeding the linearity limit, dilute the sample suitably with normal saline and repeat the assay. Apply proper dilution factor while calculation.

CALCULATION:
Activity of Alkaline Phosphatase in sample (IU/L):
= ΔA /min. x Factor

REFERENCE VALUES:

Subject	Activity at 37°C
Children (3-15 years)	250-770 IU/L
Adults	100-250 IU/L

It is recommended that each laboratory establish its own reference values.

BIBLIOGRAPHY:

Z. Klin. Chem. U. Klin. Biochem 8, 658 (1970); 10, 182 (1972).
RICK, W, Klinische chemie and Mikroskopie P. 242, 5th edition, springer vertag , Berlin (1977).

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	Attention,see instructions for use		Consult Instructions For Use
IVD	For in vitro diagnostic use only	REF	Catalog #
2°C 8°C	Store between 2-8°C	LOT	Lot Number
	Do not use if package is damaged		Date of Manufacturing
	Manufacturer		Use by