NOVIN BIO KIT

Alkaline phosphatase
p-Nitrophenylphosphate
kinetic. Liquid. IFCC

IVD *For In-Vitro diagnostic and professional use only*

2°C 8°C Store at 2-8°C

INTENDED USE

For the quantitative determination of alkaline phosphatase in human serum and heparinzed plasma.

PRINCIPLE OF THE METHOD

Alkaline phosphatase (ALP) catalyses the hydrolysis of p- nitrophenyl phosphate at pH 10.4, liberating p-nitrophenol and phosphate, according to the following reaction:

p-Nitrophenylphosphate + H_2O $\xrightarrow{ALP}$ p-Nitrophenol + Phosphate

The rate of p-nitrophenol formation, measured photometrically, is proportional to the catalytic concentration of alkaline phosphatase present in the sample

CLINICAL SIGNIFICANCE

Alkaline phosphatase is an enzyme present in almost all weaves of the organism, being particularly high in bone, liver, placenta, intestine and kidney. Both increases and decreases of plasma ALP are of importance clinically.
Causes of increased plasma ALP: Paget's disease of bone, obstructive liver disease, hepatitis, hepatotoxicity caused by drugs or osteomalacia.
Causes of decreased plasma ALP: Cretinism and vitamin C deficiency.
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R 1 Buffer	Diethanolamine (DEA) pH 10,4 mmol/L Magnesium chloride	1 0.5 mmol/L
R 2 Substrate	p-Nitrophenylphosphate (*p*NPP)	10 mmol/L

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 405 nm.
- Thermostatic bath at 25°C, 30°C ,37°C (± 0.1°C)
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

PREPARATION

Working reagent (WR)

Mix: 4 vol. (R1) Buffer + 1 vol. (R2) Substrate

Stability: 1 month at 2-8°C or 10 days at room temperature (15-25).

STORAGE AND STABILITY

- All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use.
- Do not use reagents over the expiration date.

Signs of reagent deterioration

- Presence of particles and turbidity.
- Blank absorbance (A) at 405 nm $\geq$ 1.50.

SAMPLES

Serum or heparinzed plasma. Use unhemolyzed serum, separated from the clot as soon as possible. Stability : 3 days at 2-8°C.

REFERENCE VALUES	**25°C**	**30°C**	**37°C**
Children(1-14 years)	<400 U/L	<480 U/L	<645 U/L
Adults	60-170 U/L	73-207 U/L	98-279 U/L

PROCEDURE

1. Assay conditions:

Wavelength	405nm
Cuvette	1 cm light path
Constant temperature	25 ,30 ,37°C

2. Adjust the instrument to zero with distilled water or air.

3. Pipette into a cuvette:

WR (ml)	1.2
Sample (µL)	20

4. Mix, incubate for 1 minute.

5. Read initial absorbance (A) of the sample, start the stopwatch and read absorbances at 1 min intervals thereafter for 3 min.

Calculate the difference between the covsecutive absorbances and the average absorbance differences per minute (ΔA/min)

CALCULATIONS

ΔA/min x 3300= U/L de ALP

Units: One international unit (IU) is the amount of enzyme that transforms 1µmol of substrate per minute, in standard conditions. The concentration is expressed in units per litre of sample (U/L).

Temperature conversion factors

To correct results to other temperatures multiply by

Assay temperature	Conversion factor to		
	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

QUALITY CONTROL

If control values are found outside the defined range, check the instrument, reagents and technique for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

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Adults	60-170 U/L	73-207 U/L	98-279 U/L

Factors affecting ALP activities in normal population include exercise, periods of repaid growth

in children and pregnancy.

These values are for orientation purpose; each laboratory should establish its own reference range

PERFORMANCE CHARACTERISTICS

Measuring range:

From detection limit of 0.6845 U/L to linearity limit of 1200 U/L.

If the results obtained were greater than linearity limit, dilute the sample 1/10 with NaCl 9 g/L and multiply the result by 10.

	Intra-assay (n=20)	
Mean (U/L)	174	443
SD	0.72	1.56
CV (%)	0.41	0.35
	Inter-assay (n=20)	
Mean (U/L)	175	434
SD	6.88	11.93
CV (%)	3.93	2.75

Sensitivity: 1 U/L = 0, 0003 ΔA/min.

Accuracy: Results obtained using N o v i n reagents (y) did not show systematic differences when compared with other commercial reagents (x).

The results obtained using 50 samples were the following:

Correlation coefficient $(r)^2$:0.99938. Regression equation: y= 1.025x -1.105.

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Fluoride, oxalate, citrate and EDTA inhibit alkaline phosphate activity and should therefore not be used as anticoagulants. Haemolyses interferes due to the high concentration of alkaline Phosphatase in red cells.

A list of drugs and other interfering substances with acid Phosphatase determination has been reported.

REFERENCES

1. Wenger C. et al. Alkaline phosphatase. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1094- 1098.
2. Rosalki S et al. Clin Chem 1993; 39/4: 648- 652.
3. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
4. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
5. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
6. Tietz N W et al. Clinical Guide to
7. Laboratory Tests, 3rd ed AACC 1995.

REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry