

PRODUCT CODE

CZ001

INTENDED USE

This reagent is intended for *in vitro* quantitative determination of Alkaline Phosphatase (ALP) in serum or plasma.

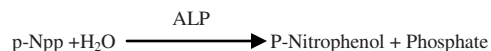
CLINICAL SIGNIFICANCE

Alkaline Phosphatase (ALP) is widely distributed throughout the body, but clinically important for diagnostic reasons are in bone, liver, placenta & intestine. Growing bone is associated with the release of ALP and so in childhood the level of ALP is around 3 times of that of adult. During pregnancy in 2nd & 3rd trimester the enzyme rises considerably due to placenta releasing ALP. It can be used to examine placental function.

Elevated levels are seen in bone diseases, E.g., pagets disease, rickets, osteoblastic metastatic & in obstructive disease of biliary tract. Decreased levels are rarely seen. E.g., in Vitamin A resistant rickets.

PRINCIPLE

Alkaline Phosphatase (ALP) catalyses the hydrolysis of P-Nitrophenyl phosphate (p-Npp) at pH 9.8, liberating p-Nitrophenyl and phosphate, according to the following reaction:



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

REAGENT COMPOSITION

ALP BUFFER R1	
Diethanolamine	1.0 mol/l
Magnesium chloride	0.5 mmol/l
ALP SUBSTRATE R2	
P-Nitrophenylphosphate	10 mmol/l

REAGENT PREPARATION

SUBSTRATE START

R1 and R2 are ready-to-use and stable until expiry date if contamination is avoided and stored at 2-8°C

SAMPLE START

Mix 4 parts of R1 + 1 Part of R2 = Mono reagent

Stability of mono reagent: 4 Weeks at 2-8°C, 5 days at 15-25°C

SPECIMEN

Serum, heparinized plasma

PRECAUTION

- The reagents contain sodium azide as preservative. Do not swallow and avoid contact with skin and mucous membranes.
- During the reaction P-Nitrophenol is produced. This is poisonous when inhaled, swallow or when absorbed through skin. If the reaction mixture comes in contact with skin or mucous membranes wash copiously with water.
- To avoid contamination, use clean laboratory wares. Avoid direct exposure of reagent to light.

ASSAY

Wavelength : 405 nm
 Cuvette : 1 cm light path
 Temperature : 25°C/ 30°C/37°C
 Measurement : Against distilled water or air

PROCEDURE:

• SUBSTRATE START

Reagent 1 Buffer	1000 µL
Sample	20 µL
Mix incubates for approx... 1 min, then add	
Reagent 2 Substrates	250 µL

• SAMPLE START

Mono reagent (R1+R2)	1000 µL
Sample	20 µL

READING FOR BOTH

Mix and read absorbance after 1 min and start stop watch. Read absorbance again after 1, 2, 3 min.

SUBSTRATE START

ALP activity U/L = $\Delta A/\text{min} \times 3433$

SAMPLE START

ALP activity U/L = $\Delta A/\text{min} \times 2757$

Linearity

The reagent is linear up to 700 U/L

If the activities exceed 700 U/L, mix 50 µL of sample with 200µL of 0.9% NaCl solution and multiply the result by 5.

Interferences:

Fluoride, oxalate, citrate and EDTA inhibit alkaline phosphate activity and should therefore not be used as anticoagulants. Hemolysis 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 by Young et.⁵

NORMAL RANGE















	25°C	30°C	37°C
Women U/L	40-190	48-223	64-306
Men U/L	50-190	60-223	80-306
Children up to 15 years U/L,	up to 400	up to 488	up to 644

Each laboratory should establish reference ranges for its own patients' population.

QUALITY CONTROL

All control sera with values determined by this method can be used.

SYMBOL ON LABELS

Symbols	Signify	Symbols	Signify
	Catalogue Number		Pack Size
	Expiry Date		Volume
	Storage Condition		Lot Number
	Instruction for Use		In Vitro Diagnostics
	Manufacturing Date		Manufacturer
	Number of Tests		For Single Use Only
	EC Representative		European conformity

BIBLIOGRAPHY

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