Alkaline Phosphatase
Kinetic-DGKC Method

IVD CE

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:

$$\text{p-Npp} + H_2O \xrightarrow{\text{ALP}} \text{P-Nitrophenol} + \text{Phosphate}$$

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
1- The reagents contain sodium azide as preservative. Do not swallow and avoid contact with skin and mucous membranes.
2- 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.
3- 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 = ΔA/min. X 3433

SAMPLE START
ALP activity U/L = ΔA/min. X 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
REF	Catalogue Number	SIZE	Pack Size
	Expiry Date	VOL	Volume
	Storage Condition	LOT	Lot Number
	Instruction for Use	IVD	In Vitro Diagnostics
	Manufacturing Date		Manufacturer
	Number of Tests	2	For Single Use Only
EC REP	EC Representative	CE	European conformity

BIBILOGRAPHY

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Bio Research For Medical Diagnostics
Muslim Al Attar Street,P.O.Box:1235,
Amman-11953,Jordan
Tel:+962 64892525, Fax: +962 64892526,
www.bioresearch.com.jo

EC REP

MDSS GmbH
Schiffgraben 41
30175 Hannover, Germany