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ALKALINE PHOSPHATASE (ALP) CE

STABLE LIQUID REAGENT

Cat. No.::	48261	48263	48262
	125 ml	10x25 ml	600 ml
	(1x100ml+1x25ml)	(10x20ml+10x5ml)	(1x480ml+1x120ml)

Reagent kit for the quantitative determination of alkaline phosphatase activity in serum, DGKC method.

Alkaline phosphatase is a membrane-bound enzyme which is present in most tissues. It has three different isoenzymes derived from small intestine-placenta-bone/liver/kidney. It is a dimer molecule containing Zn^{++} ions, which play a role in the maintenance of structure and catalysis. The enzyme found in human serum is derived from bone, liver and small intestine. During pregnancy the enzyme from the placenta dominates (it is heat stable at 65°C). In the past the isoenzymes were separated using various inhibitors and heat. The role of electrophoresis is growing in determining the concentrations. The increase in enzyme activity is prevalent in various hepatic and bone decrease states. The level is also increased in certain diseases of the thyroid gland, intestinal tract and in several bacterial infection.

Principle
The enzyme catalyses the hydrolysis of monophosphates at an alkaline pH. In the past various substrates were used (including glycerophosphate, phenylphosphate), according to the recommendation by DGKC which is a kinetic method. The Alkaline phosphatase present in the sample catalyses the hydrolysis of p-Nitrophenylphosphate (pNPP) during which p-Nitrophenol and Phosphate are released. Mg^{++} ions enhance activity. The increase in absorbance at 405 nm correlates with the activity of serum alkaline phosphatase.
Kinetic determination of the alkaline phosphatase based upon DGKC and SCE Recommendation (p-NPP).

$$p\text{-nitrophenylphosphate} + H_2O \xrightarrow{ALP+Mg^{++}} p\text{-nitrophenol} + \text{inorganic phosphate}$$

Reference values
Children: **180-1200 U/l (3,00-20,0 µkat/l)**
Adults: **98-279 U/l (1,63-4,65 µkat/l)**
It is recommended that each laboratory should assign its own normal range.

Reagents
1. Reagent (R1)
Diethanolamine buffer, pH=9.80 1 mol/l
Magnesium chloride 0.6 mmol/l
2. Reagent (R2)
p-Nitrophenylphosphate (solution) 10 mmol/l

Safety instructions:
Reagent 1:
X, Harmful
R22 Harmful if swallowed
R38 Irritating to skin.
R41 Risk of serious damage to eyes
R48/22 Harmful: danger of serious damage to health by prolonged exposure if swallowed.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37/39 Wear suitable protective clothing, gloves and eye/face protection
S46 If swallowed, seek medical advice immediately and show this container or label.

Samples

Serum free of haemolysis.

PROCEDURE

The reagents are ready to use
Assay conditions
Wavelength: 405-410 nm
Temperature: 37 °C
Cuvette: 1 cm light path
Read against: distilled water or air
Method: kinetic (increasing)

• **Two-reagent procedure**

Reagent 1	800 µl
Sample	20 µl

Mix and wait 1 minute.

Reagent 2	200 µl

Mix and after a 60-second incubation read the change of optical density (ΔA) during 2 minutes. Determine the change of optical density per minute (ΔA/min).

Calibration: (37 °C, DGKC method, DEA puffer)
S1: Distilled water
S2: Roche C.F.A.S. (Calibrator for automated system) or
Randox Calibration Serum Level I or
Randox Calibration Serum Level II
Calibration frequency
Two-point calibration is recommended:
- after reagent lot change,
- as required following quality control procedures.

Calculation using calibration

$$\frac{\Delta A_{sample}}{\Delta A_{standard}} x C_{standard} = C_{sample}$$

A = Absorbance, C = Concentration

Calculation using factor

405 nm: ΔA/minute x 3250 = U/l

405 nm: ΔA/minute x 54,2 = µkat/l

Quality control
A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

PERFORMANCES DATA

The following data were obtained using the Olympus 600 analyzer.

Linearity
The method is linear up to 1800 U/l (30,0 µkat/l)

Sensitivity
It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.001 Abs units/min is equivalent to 3.00 U/l (0,05µkat/l) Alkaline-phosphatase activity at 405 nm.

Precision

	Reproducibility		
Sample	Average activity (U/l)	SD	CV%
Sample I.	176	4.03	2.29
Sample II.	416	5.95	1.43

	Repeatability		
Sample	Average activity (U/l)	SD	CV%
Sample I.	191	1.75	0.91
Sample II.	642	6.17	0.96

Correlation
Comparative studies were done to compare our reagent with another commercial alkaline-phosphatase assay on 47 human samples. The alkaline phosphatase activity was between 36 U/l and 1925 U/l.
The results from these studies are detailed below.
Correlation coefficient: r=0.9998
Linear regression: y (U/l)= 0.998x+3.949
(x= other commercial reagent, y= own reagent).

Specificity
Bilirubin 128.3 µmol/l (7,5mg/dl), lipid 1000mg/dl, glucose 55.5 mmol/l (1000mg/dl) and ascorbic acid 2.84 mmol/l (50mg/dl) don't interfere with the assay up to the given levels.

Note

The enzyme activity is best measured within a few hours of taking the blood sample. Do not pipette reagents by mouth! The stability of the isoenzymes is different. Chelating agents (EDTA) interfere with the reaction.

Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solutions and reagents described above for any purpose other than described herein.

For *in vitro* diagnostic use only.

The following symbols are used on labels

IVD For in vitro diagnostic use

Use by (last day of the month)

Temperature limitation

LOT Batch Code

REF Code

Bibliography
Haussament T.U. et. al. Clin. Chem. Acta 35, 271-273 (1977)