

Alkaline Phosphatase (ALP) Reagent

2 Part Liquid

PRODUCT SUMMARY

Stability	:	Until Expiry at 2-8°C
Linear Range	:	Up to 2000 U/L (33.3 µkat/L)
Specimen Type	:	Serum or Plasma
Method	:	Kinetic
Reagent Preparation	:	Supplied ready to use.

IVD

INTENDED USE

This reagent is intended for the in vitro quantitative determination of ALP (Orthophosphoric - Monoester Phosphohydrolase, Alkaline Optimum, EC 3.1.3.1), in human serum or plasma on both manual and automated systems.

CLINICAL SIGNIFICANCE¹

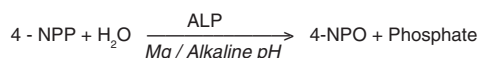
Human ALP consists of a group of enzymes (at least 5) which hydrolyse phosphates at an alkaline pH (6-8). ALP is found in practically all tissues of the body but in high concentrations in the osteoblasts of bone, liver, placenta, kidney, intestinal wall and lactating mammary glands. In adults the ALP normally found circulating in the serum is largely derived from the liver. In children or in adolescents going through pubertal growth spurts there is an additional contribution from bone and this accounts for the higher reference interval for these groups. Pregnancy also raises the normal values of ALP.

Raised ALP levels are often observed in bone disease or liver disease involving the biliary tract. If the source of the isoenzyme is not apparent then estimation of GGT may help differentiate between the two. A raised GGT in the presence of a raised ALP would suggest the liver is the primary source.

Increased ALP (usually normal GGT) is seen in Osteomalacia and Rickets, primary hyperparathyroidism with bone involvement, Pagets disease, secondary carcinoma in bone and some cases of osteogenic sarcoma. Increased levels of ALP (usually with a raised GGT) is seen in cholestasis, hepatitis, cirrhosis, space occupying lesions and malignancy with bone or liver involvement or direct production. Low levels of ALP may be observed in conditions which cause arrested bone growth or in hypophosphatasia.

METHODOLOGY

This alkaline phosphatase (ALP) method is based on the recommendations of the International Federation of Clinical Chemistry (IFCC)². This method utilises 4-nitrophenylphosphate as the substrate. Under the optimised conditions ALP present in the sample catalyses the following transphosphorylation reaction.



At the pH of the reaction 4-nitrophenoxide has an intense yellow color. The reagent also contains a metal ion buffer system to ensure that optimal concentrations of Zinc and Magnesium are maintained. The metal ion buffer can also chelate other potentially inhibitory ions which may be present. The reaction is monitored by measuring the rate of increase in absorbance at 405 nm which is proportional to the activity of ALP in the serum.

Abbreviations:

4-NPP	=	4-Nitrophenylphosphate
4-NPO	=	4-Nitrophenoxide
AMP	=	2-Amino-2-methyl-1-propanol
ALP	=	Alkaline phosphatase

REAGENT COMPOSITION

Active Ingredients

Reagent 1:

	Concentration
AMP	446 mmol/L
Mg Acetate	2.55 mmol/L
ZnSO ₄	1.275 mmol/L
HEDTA	2.55 mmol/L

Reagent 2:

4 - NPP	81.6 mmol/L
pH 10.75 ± 0.10 at 20°C	

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorized Representative	Temperature Limitation	
IVD	For in vitro diagnostic use	Use by/Expiration Date	
LOT	Batch code/Lot number	CAUTION. CONSULT INSTRUCTIONS FOR USE.	
REF	Catalogue number	Manufactured by	
Consult instructions for use			
REAG 1	Reagent 1 (R1)	REAG 2	Reagent 2 (R2)

WARNING: Do not ingest. Avoid contact with skin and eyes. If spilt thoroughly wash affected areas with water. Reagent contains sodium azide which may react with copper or lead plumbing. Flush with plenty of water when disposing. For further information consult the Alkaline Phosphatase (ALP) 2 Part Liquid Reagent Material Safety Data Sheet.

REAGENT PREPARATION

Reagents supplied ready to use.

STABILITY AND STORAGE

Prior to use:

When stored between 2-8°C the reagents are stable until the expiration date stated on the bottle and kit box label.

Once the Reagent is Opened:

When stored capped at 2-8°C, the reagents are stable until the expiration date stated on the bottle and kit box label.

Combined reagent is stable for at least 5 days when stored at 2 -8°C.

Indications of Reagent Deterioration:

- Turbidity;
- Reagent absorbance >1.15 at 405nm (1cm lightpath); and/or
- Failure to recover control values within the assigned range.

SPECIMEN COLLECTION AND HANDLING²

Serum: Use non - haemolysed serum.

Plasma: Use heparin. Do not use EDTA, Oxalate or Fluoride.

Storage: ALP activity in stored serum increases with time. The increase in activity of stored serum at room temperature is minimal for up to 4 hours. The increase in activity of serum stored at 2-8°C or frozen sera upon warming is significant. The rate of this increase is both temperature and time dependent.

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- A clinical chemistry analyser capable of maintaining constant temperature (37°C) and measuring absorbance at 405 nm
- Analyser specific consumables, eg: sample cups.
- Assayed Normal and Abnormal control material.

ASSAY PROCEDURE

The following system parameters are recommended. Individual instrument applications are available on request from the Technical Support Group.

SYSTEM PARAMETERS

Temperature	30/37°C
Wavelength	405 nm (405-420 nm)
Secondary Wavelength	660nm (600 - 660nm)
Assay Type	Rate/Kinetic
Direction	Increase
Sample : Reagent Ratio	1 : 40 (R1): 10 (R2)
eg: Sample Vol	5 µL
Reagent 1 Vol	200 µL
Reagent 2 Vol	50 µL
Delay Time (sample + R1)	5 minutes
Lag Time (sample+R1+R2)	30 seconds
Read Time	2 minutes
Reagent Blank Limits	Low 0.0 AU
(405nm, 1cm lightpath)	High 1.15 AU
Linearity	Up to 2000 U/L
(refer to linearity section)	(33.3 µkat/L)
Analytical Sensitivity	0.37 ΔmA/min per U/L
(405nm, 1cm lightpath)	(22.2 ΔmA/min per µkat/L)

CALCULATIONS

Results are calculated, usually automatically by the instrument, as follows:

Activity in U/L = $\Delta\text{Abs}/\text{min} \times \text{Factor}$

$$\text{Factor} = \frac{\text{TV} \times 1000}{18.8 \times \text{SV} \times \text{P}}$$

Where:

TV = Total reaction volume in mL
SV = Sample volume in mL
18.8 = millimolar absorption coefficient of 4-nitrophenol at 405nm
P = Cuvette pathlength in cm

Example:

$\Delta\text{Abs}/\text{min}$ = 0.038
Factor = 2713
ALP = $0.038 \times 2713 = 103 \text{ U/L}$

NOTES

- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- Valid results depend on accurately calibrated instruments, timing and temperature control.
- Unit conversion: $\text{U/L} \times 16.67 \times 10^{-3} = \mu\text{kat/L}$.

CALIBRATION

Not required. The rate of reaction is converted to U/L of activity by a calculation factor. Refer to the calculation section of this package insert.

QUALITY CONTROL

To ensure adequate quality control, normal and abnormal controls with assayed values should be run as unknown samples:-

- At least once per day or as established by the laboratory.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.

Control results falling above the upper limit or below the lower limit of the established range indicates the assay may be out of control. The following corrective actions are recommended in such situations:-

- Repeat the same controls.
- If repeated control results are still outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact Technical Services or your local distributor.

LIMITATIONS

- Studies to determine the level of interference from haemoglobin, bilirubin and lipaemia were carried out. The following results were obtained:
Haemoglobin: No interference from haemoglobin up to 790mg/dL.
Free bilirubin: No interference from bilirubin up to 278 $\mu\text{mol/L}$ (16.3 mg/dL).
Conjugated Bilirubin: No interference from bilirubin up to 290 $\mu\text{mol/L}$ (17.1 mg/dL).
Lipaemia: When measured bichromatically no interference from lipaemia, measured as Triglycerides, up to 22.8 mmol/L (2000 mg/dL).
- Young DS³ has published a comprehensive list of drugs and substances which may interfere with this assay.

EXPECTED VALUES⁴

At 37°C	4-15 Y	Males:	54 - 369 U/L	(0.902 - 6.16 $\mu\text{kat/L}$)
		Females:	54 - 369 U/L	(0.902 - 6.16 $\mu\text{kat/L}$)
	20-50 Y	Males:	53 - 128 U/L	(0.885 - 2.13 $\mu\text{kat/L}$)
		Females:	42 - 98 U/L	(0.701 - 1.63 $\mu\text{kat/L}$)
	≥60 Y	Males:	56 - 119 U/L	(0.935 - 1.98 $\mu\text{kat/L}$)
		Females:	53 - 141 U/L	(0.885 - 2.35 $\mu\text{kat/L}$)

The quoted values are representative of the expected range for this method and should serve as a guide only. It is recommended that each laboratory verify this range or derives a reference interval for the population that it serves.⁵

PERFORMANCE DATA

The following performance data was obtained using the ALP 2 part Liquid reagent on an automated clinical chemistry analyser. Users should establish product performance on their specific analyser used.

IMPRECISION

Imprecision was evaluated using two levels of commercial control and following the NCCLS EP5-T procedure.⁶

Within Run:	LEVEL I	LEVEL II
Number of data points	80	80
Mean (U/L)	74	319
Mean ($\mu\text{kat/L}$)	1.24	5.33
SD (U/L)	0.65	2.02
SD ($\mu\text{kat/L}$)	0.011	0.034
CV (%)	0.9	0.6

Total:	LEVEL I	LEVEL II
Number of data points	80	80
Mean (U/L)	74	319
Mean ($\mu\text{kat/L}$)	1.24	5.33
SD (U/L)	2.58	9.34
SD ($\mu\text{kat/L}$)	0.043	0.156
CV (%)	3.5	2.9

METHOD COMPARISON

Comparison studies were carried out using another similar commercially available method. Serum and plasma (Heparin) samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained:

Number of samples	60
Range of sample results	48 - 225U/L (0.802 - 3.76 $\mu\text{kat/L}$)
Mean of reference method results	100 U/L (1.67 $\mu\text{kat/L}$)
Mean of ALP results	105 U/L (1.75 $\mu\text{kat/L}$)
Slope	1.03
Intercept	-0.33 (-0.006 $\mu\text{kat/L}$)
Correlation coefficient	0.9995

LINEARITY

When run as recommended the assay is linear up to 2000 U/L (33.3 $\mu\text{kat/L}$).


ANALYTICAL SENSITIVITY

When run as recommended the sensitivity of the assay is 0.37 $\Delta\text{mA}/\text{min}$ per U/L (22.2 $\Delta\text{mA}/\text{min}$ per $\mu\text{kat/L}$).

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REF

Reorder Information

Catalogue No.

	REAG 1	REAG 2
TR11320	1 x 125 mL	1 x 35 mL
TH11301 (Hitachi)	4 x 80 mL	4 x 20 mL
TL11301 (iLab 600)	5 x 80 mL	5 x 20 mL
TY11301 (Hitachi)	4 x 50 mL	4 x 14.5 mL
7500-103A	4 x 500 mL	
7500-203A		2 x 250 mL