

# Alkaline Phosphatase (ALP) Reagent

## AMP Buffer (IFCC)

### PRODUCT SUMMARY

Stability	:	30 days at 2-8°C
Linear Range	:	Up to 1000 U/L (16.7 µkat/L)
Specimen Type	:	Serum or plasma
Method	:	Kinetic
Reagent Preparation	:	Add specified volume of buffer.

### 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 A	Reagent A	REAG B	Reagent B

### 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.

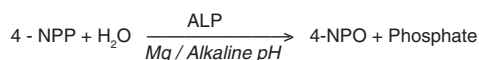
### CLINICAL SIGNIFICANCE<sup>1</sup>

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)<sup>2</sup>. 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 colour. 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 A:	Concentration
4-NPP	16.3 mmol/L
Reagent B:	
AMP	420 mmol/L
Mg Acetate	2.4 mmol/L
ZnSO <sub>4</sub>	1.2 mmol/L
HEDTA	2.4 mmol/L
pH 10.7 ± 0.1 at 20°C	

**WARNING:** Do not ingest. Avoid contact with skin and eyes. If spilt thoroughly wash affected areas with water. For further information consult the ALP-AMP (IFCC) Reagent Material Safety Data Sheet. **The Packaging of This Product Contains Dry Natural Rubber.** Exercise precaution when handling metal crimps and broken glass vials, as sharp edges can injure the user.

### REAGENT PREPARATION

Reconstitute Reagent A with the volume of buffer, Reagent B, indicated on the vial label. Mix gently until dissolved.

### STABILITY AND STORAGE

#### Prior to use:

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

#### Reconstituted Reagent:

When stored capped at 2-8°C, the reagent is stable for at least 30 days.

#### Indications of Reagent Deterioration:

- Turbidity,
- Absorbance > 0.8 at 405nm (1cm); and/or
- Failure to recover control values within the assigned range.

### SPECIMEN COLLECTION AND HANDLING<sup>2</sup>

**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

- If required, pipettes for accurately dispensing measured volumes.
- A clinical chemistry analyser capable of maintaining constant temperature (37°C) and measuring absorbance at 405nm.
- Analyser specific consumables, eg: sample cups.
- Normal and Abnormal control material.

### ASSAY PROCEDURE

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

### SYSTEM PARAMETERS

Temperature	30/37°C
Primary Wavelength	405 nm (405 - 420nm)
Secondary Wavelength	660 nm (600 - 660nm)
Assay Type	Rate / Kinetic
Direction	Increase
Sample:Reagent ratio	1:50
e.g. Sample vol	4 µL
Reagent vol	200 µL
Lag Time	60 seconds
Read Time	120 seconds
Reagent Blank Limits	Low 0.0 AU
(405nm, 1cm lightpath)	High 0.8 AU
Linearity	Up to 1000 U/L
(refer to Linearity section)	(16.7 µkat/L)
Analytical Sensitivity	0.37 ΔmA/min per U/L
(405nm, 1cm lightpath)	(22 ΔmA/min per µkat/L)

## CALCULATIONS

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

**Activity in U/L =  $\Delta$ Abs/min x 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:

$\Delta$ Abs/min = 0.075  
Factor = 2713  
ALP = 0.075 x 2713 = 203 U/L

## NOTES

- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- If the change in absorbance is greater than 0.37 AU/min, dilute with saline and reassay. Multiply the final result by the dilution factor.
- Valid results depend on accurately calibrated instruments, timing and temperature control.
- Unit Conversion: U/L x 16.67 x 10<sup>-3</sup> =  $\mu$ 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 control 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 outside the upper or lower limits of the established ranges indicate 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 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 Support or the local distributor.

## LIMITATIONS

- Studies to determine the level of interference from haemoglobin, bilirubin and lipaemia were carried out on a well maintained automated clinical chemistry analyser. The following results were obtained:  
**Haemoglobin:** No interference from haemoglobin up to 500 mg/dL.  
**Bilirubin:** No interference from bilirubin up to 340  $\mu$ mol/L (20 mg/dL).  
**Lipaemia:** No interference from lipaemia, measured as triglycerides, up to 4 mmol/L (360 mg/dL).
- Young DS<sup>3</sup> has published a comprehensive list of drugs and substances which may interfere with this assay.

## EXPECTED VALUES

At 37°C	20-50 Y	Males:	53 - 128 U/L (0.90 - 2.13 $\mu$ kat/L)
		Females:	42 - 98 U/L (0.70 - 1.63 $\mu$ kat/L)
	$\geq$ 60 Y	Males:	56 - 119 U/L (0.93 - 1.98 $\mu$ kat/L)
		Females:	53 - 141 U/L (0.90 - 2.35 $\mu$ kat/L)
At 30°C	20-50Y	Males:	30 - 90 U/L (0.50 - 1.50 $\mu$ kat/L)
		Females:	20 - 80 U/L (0.33 - 1.33 $\mu$ kat/L)
	$\geq$ 60 Y	Males:	30 - 90 U/L (0.50 - 1.50 $\mu$ kat/L)
		Females:	40 - 110 U/L (0.67 - 1.85 $\mu$ 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.<sup>4</sup>

## PERFORMANCE DATA

The following data was obtained using the ALP-AMP (IFCC) Reagent on a well maintained 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<sup>5</sup>.

Within run:	LEVEL I	LEVEL II
Number of Data Points	80	80
Mean (U/L / $\mu$ kat/L)	94 / 1.57	425 / 7.08
SD (U/L / $\mu$ kat/L)	1.9 / 0.032	5.0 / 0.083
CV (%)	2.0	1.2

Total:	LEVEL I	LEVEL II
Number of Data Points	80	80
Mean (U/L / $\mu$ kat/L)	94 / 1.57	425 / 7.08
SD (U/L / $\mu$ kat/L)	3.6 / 0.060	11.9 / 0.198
CV (%)	3.9	2.8

## 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 sample pairs	60
Range of sample results	13 - 1241 U/L (0.217 - 20.7 $\mu$ kat/L)
Mean of reference method results	110 U/L (1.83 $\mu$ kat/L)
Mean of ALP-AMP results	125 U/L (2.08 $\mu$ kat/L)
Slope	1.16
Intercept	-2.24 U/L (-0.037 $\mu$ kat/L)
Correlation coefficient	1.000

## LINEARITY


When run as recommended the assay is linear up to 1000 U/L (16.7  $\mu$ kat/L).

## ANALYTICAL SENSITIVITY

When run as recommended the sensitivity of the assay is 0.37  $\Delta$ mA/min per U/L (22  $\Delta$ mA/min per  $\mu$ kat/L)

## REFERENCES

- Zilva JF, Pannall PR, "Plasma Enzymes in Diagnosis" in Clinical Chemistry in Diagnosis and Treatment. Lloyd London 1979: Chap 15 343.
- IFCC method for the measurement of ALP J Clin Chem Clin Biochem 1983: 21:731-48.
- Young DS. Effects of Drugs on Clinical Laboratory Tests. Third Edition 1990: 3: 19-25.
- Wachtel M et al, Creation and Verification of Reference Intervals. Laboratory Medicine 1995; 26:593-7.
- Kennedy JW et al: User Evaluation of Precision Performance of Clinical Chemistry Devices 1989: NCCLS Vol 4, No 8: 185-95.

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**REF**

### Reorder Information

Catalogue No.	REAG A	REAG B
TR11110/1140-200	20 x 10 mL	1 x 200 mL
TR11115	20 x 20 mL	2 x 200 mL
TR11103/1140-500	10 x 50 mL	1 x 500 mL
TR11104	10 x 200 mL	4 x 500 mL