



# ALKALINE PHOSPHATASE KIT (DEA)

(pNPP Kinetic Method)

(For veterinary invitro diagnostic use only)

## INTENDED USE

QUADRAPED™ Alkaline Phosphatase kit is used for the determination of Alkaline Phosphatase Activity in serum.

## SUMMARY

Alkaline Phosphatase (ALP) is an enzyme of the Hydrolase class of enzymes and acts in an alkaline medium. It is found in high concentrations in the liver, biliary tract epithelium and in the bones. Normal levels are age dependent and increase during bone development. Increased levels are associated mainly with liver and bone disease. Moderate increases are seen in Hodgkins disease and congestive heart failure.

## PRINCIPLE

ALP at an alkaline pH hydrolyses p-Nitrophenylphosphate to form p-Nitrophenol and Phosphate. The rate of formation of p-Nitrophenol is measured as an increase in absorbance which is proportional to the ALP Activity in the sample.

ALP

p-Nitrophenylphosphate → p-Nitrophenol + Phosphate

## EXPECTED VALUES

Species	Alkaline Phosphatase (U/L)
Dog	1 - 114
Cat	0 - 45
Cow	22 -140
Horse	100 -300
Pig	118 -395
Sheep	68 - 387
Goat	93 - 387
Rabbit	12 - 96
Buffalo	50 -150

It is recommended that each laboratory establish its own range as reference ranges may vary between laboratories.

## PRESENTATION

REF	1126020103
Pack Size	10 x 3 ml
L1 Buffer Reagent	35 ml
T1 Substrate Tablets	10 Nos.

## COMPOSITION

DEA Buffer 1M; pH 10.3; Magnesium Chloride 0.5mM; pNPP 10mM; Non Reactive Stabilizers and Preservatives.

## STORAGE / STABILITY

Contents are stable at 2-8°C till the expiry mentioned on the labels.

## REAGENT PREPARATION

**Working Reagent:** Dissolve 1 Substrate Tablet in 3.2 ml (10 x 3 ml pack) of Buffer Reagent.

This working reagent is stable for at least 15 days when stored at 2-8°C.

The substrate is light & temperature sensitive. Take adequate care, especially after reconstitution.

## SAMPLE MATERIAL

Serum. Free from hemolysis. ALP is reported to be stable in serum for 3 days at 2-8°C.

## SAMPLE WASTE AND DISPOSAL

Do not reuse the reagent containers, bottles, caps or plugs due to the risks of contamination and the potential to compromise reagent performance.

Appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.

Handle specimens, solid and liquid waste and test components in accordance with local regulations and NCCLS guidelines M29, or other published biohazard safety guideline

## MATERIALS REQUIRED BUT NOT PROVIDED

Photometer analyzer with standard thermostatic cuvette holder, micropipette and appropriate laboratory equipment.

## PROCEDURE

Wavelength / filter : 405 nm  
 Temperature : 37° C / 30° C / 25° C  
 Light path : 1 cm

Pipette into a clean dry test tube labelled as Test (T):

Addition Sequence	(T) (ml)
Working Reagent	1.0
Incubate at the assay temperature for 1 min. and add	
Sample	0.02

Mix well and read the initial absorbance  $A_0$  after 30 secs. & repeat the absorbance reading after every 1 & 2 mins. Calculate the mean absorbance change per min. ( $\Delta A / \text{min}$ ).

## CALCULATIONS

ALP Activity in U/L =  $\Delta A / \text{min} \times 2754$

## QUALITY CONTROL

The following process is recommended for QC during the assay of Alkaline Phosphatase. \*Define and establish acceptable range for your laboratory.

- Two levels of control (Normal and Abnormal) are to be run on a daily basis.
- If QC results fall outside acceptance criteria, re-calibration may be necessary.
- Review QC results and run acceptance criteria following a change of reagent lot.

## SPECIFIC PERFORMANCE CHARACTERISTICS

LOD : 3 U/L  
 LOQ : 28 U/L  
 Lower Limit : 3 U/L  
 Higher Limit : 700 U/L

If the absorbance change ( $\Delta A / \text{min}$ ) exceeds 0.250, use only the value of the first 2 mins. to calculate the results, or dilute the sample 1+9 with normal saline (NaCl 0.9%) and repeat the assay (Results x 10)

## Interferences:

Sample when spiked with interferent such as upto 20mg/dl bilirubin, 1000mg/dl intralipid does not affect the ability of the kit to determine the Alkaline Phosphatase concentration.

## Precision:

### Within run

Within run	n	Mean	SD	% CV
Sample 1	10	255	1.59	0.62
Sample 2	10	455	1.74	0.38
Sample 3	10	141	1.95	1.39

### Between run

Between run	n	Mean	SD	% CV
Sample 1	10	254	1.74	0.68
Sample 2	10	454	1.74	0.38
Sample 3	10	146	1.95	1.34

## Method comparison:

Comparative studies were done to compare our reagent with another commercial Alkaline Phosphatase Assay. No significant differences were observed. Details of the comparative studies are available on request.

## NOTE

In vitro diagnostic reagent for laboratory and professional use only Not for medicinal use. The reagent contain sodium azide 0.1% as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water. Only clean and dry glassware must be used. Samples having a very high activity show a very high initial absorbance. If this is suspected then dilute the sample and repeat the assay. Do not use turbid, deteriorated or leaking reagents.

## REFERENCES

- Bowers, G. N. McCommb, R.B. (1972) Clin. Chem. 18:97.
- Recommendations of German Society for Clinical Chemistry, (1972) Z. Clin. Chem. Bio. 10: 182.
- Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology, Kenneth S. Latimer, ISBN Jane Wardrop, 6th Edition - 2010.
- Clinical Biochemistry of Domestic Animals, Sixth Edition, 2008 by Kaneko J.J., Harvey J.W. & Bruss M.L.
- Data on file: Coral Clinical Systems.

## System Parameters

Reaction	: Kinetic	Interval	: 30 Sec.
Wavelength	: 405 nm	Sample Vol.	: 0.02 ml
Zero Setting	: Distilled Water	Reagent Vol.	: 1.00 ml
Incub. Temp.	: 37° C	Standard	: ---
Incub. Time	: ---	Factor	: 2754
Delay Time	: 30 Sec.	React. Slope	: Increasing
Read Time	: 120 Sec.	Linearity	: 700 U/L
No. of read.	: 4	Units	: U/L

Store at 2-8°C	Manufacturer	In vitro Diagnostic Medical Device	Buffer Reagent	pNPP Kinetic Method
Use by (Last day of stated month)	Consult Instructions for use	Batch Number	Substrate Tablets	This way up
Date of Manufacture	Catalogue Number			

QALP/10625/VER-01



Manufactured by:

## Coral Clinical Systems

A Division of Tulip Diagnostics (P) Ltd.

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