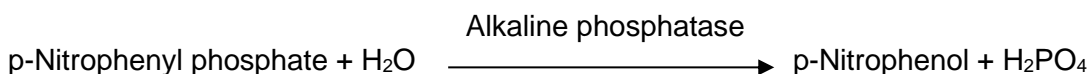


<b>Originating Department</b>	QC
<b>Approval Departments</b>	QA, QC
<b>Approval Date</b>	31 <sup>st</sup> March 2017
<b>Effective Date</b>	19 <sup>th</sup> April 2017

## 1.0 PRODUCT DETAILS

- 1.1 **Enzyme Name:** Alkaline phosphatase
- 1.2 **Systematic Name:** Orthophosphoric-monoester phosphohydrolase (alkaline optimum)
- 1.3 **E.C. Number:** 3.1.3.1
- 1.4 **Source:** Bovine intestinal mucosa

## 2.0 ASSAY PRINCIPLE



The change in optical density at 405nm per unit time is a measure of the Alkaline phosphatase activity.

## 3.0 UNIT DEFINITION

That amount of enzyme causing the hydrolysis of one micromole of p-Nitrophenyl phosphate per minute at pH 9.8 and 37°C.

## 4.0 EQUIPMENT REQUIRED

Double beam UV/vis spectrophotometer, with recorder and temperature control, set at 37°C (± 0.2°C).  
Water bath at 37°C (± 0.2°C).  
Disposable cuvettes, disposable test tubes, glass test tubes, glass pipettes and automatic pipettes.

## 5.0 REAGENTS REQUIRED

When using the following reagents please refer to the manufacturer's instructions for safe handling and disposal.

### Reagent details

Magnesium chloride hexahydrate ( $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ) AnalaR  
Supplier: VWR International  
Product No.: 10149  
F.W.: 203.3

p-Nitrophenyl phosphate disodium hexahydrate  
Supplier: Sigma Chemical Company Ltd.  
Product No.: P 4744  
F.W.: 371.1

Hydrochloric acid (6M)  
Supplier: Sigma  
Product No.: P5710V

Diethanolamine (Bis(2-hydroxyethyl)amine; 2,2'iminodiethanol), >99.5%  
Supplier: Fluka Chemicals Ltd.  
Product No.: 31589  
F.W.: 105.14

## 6.0 PREPARATION OF REAGENTS

### 6.1 1M Magnesium chloride solution (Reagent 1)

Dissolve 4.06g of Magnesium chloride in approximately 16ml of analytical grade water and then make up to 20ml with the same. Stable at 5°C for 2 weeks.

### 6.2 Hydrochloric acid (6M) (Reagent 2)

Use as required and refer to the manufacturer's expiry date.

### 6.3 Diluent buffer (1M Diethanolamine/0.5mM Magnesium chloride, pH 9.8 at 37°C) (Reagent 3)

Dissolve 52.57g of Diethanolamine in approximately 400mls of analytical grade water and add 0.25ml of 1M Magnesium chloride (reagent 1). Heat the solution to 37°C ( $\pm 0.2^\circ\text{C}$ ) then adjust the pH to 9.8 with 6M hydrochloric acid (reagent 2). Make up to 500ml with analytical grade water and store in a dark bottle. This solution must be prepared daily.

### 6.4 Substrate (approximately 0.67M p-Nitrophenyl phosphate) (Reagent 4)

Dissolve 247mgs of p-Nitrophenyl phosphate in 1ml of analytical grade water. Store in a dark bottle and prepare fresh daily.

### 6.5 Enzyme solution

Dilute to approximately 0.15U/ml in diluent buffer.

## 7.0 TEST PROCEDURE

Temperature = 37°C    Wavelength = 405nm    Light path = 1cm

Into disposable test tubes at 37°C pipette the following:

	TEST	REF
Diluent buffer (reagent 3)	2.90ml	2.95ml
Substrate solution (reagent 4)	0.05ml	0.05ml
Allow to equilibrate for approx. 5 minutes then add:		
Enzyme solution	<u>0.05ml</u>	<u>0.00ml</u>
Total reaction mix volume (Vt) =	<u>3.00ml</u>	<u>3.00ml</u>

Transfer to disposable cuvettes and record the increase in absorbance at 405nm, reading the test solution versus the reference solution, for approximately 5 minutes. Measure the change in absorbance per minute over the linear portion of the curve and use this value in the calculation.

## 8.0 CALCULATION

$$\text{Volume activity (U/ml)} = \frac{\Delta E_{405\text{nm}}/\text{min} \times V_t}{V_s \times \epsilon} \times \text{dilution factor}$$

Where:

$$V_t = \text{final volume of reaction mix (ml)} = 3.0$$

$$V_s = \text{sample volume (ml)} = 0.05$$

$$\epsilon = \text{micromolar extinction coefficient for p-Nitrophenol-phosphate (cm}^2/\mu\text{mole)} = 18.2$$

$$\text{Volume activity (U/ml)} = \Delta E_{405}/\text{min} \times 3.30 \times \text{dilution factor}$$

$$\text{Specific activity (U/mg protein)} = \frac{\text{U/ml}}{\text{mg protein / ml}} \quad (\text{Liquid products})$$

$$\text{Specific activity (U/mg protein)} = \frac{\text{U/mg}}{\text{mg protein / mg}} \quad (\text{For freeze-dried powders})$$

## 9.0 PROTEIN DETERMINATION

Protein concentration is determined by the Biuret method, Procedure No, AP99 (Analytical Procedures Manual).

## 10.0 E<sup>1%</sup> DETERMINATION 280

The E<sup>1%</sup> is determined according to Proc. No. AP63 (Analytical Procedures Manual).  
280

## 11.0 REFERENCES

1. E.Moessner, M. Boll, G. Pfeleiderer, *Hoppe-Sayeler's Z. Physiol. Chem.* Vol. 361, page 543-549, April 1980.

## 12.0 REVISION HISTORY

Document Issue Number	Section Number	Summary of Changes
03	Global	Header and footer updated to current format.
	1.5	Section removed as no longer required.
	5.0	Change of molarity and Supplier for HCl.
	6.0	Paragraph amended for preparing Hydrochloric Acid to read, 'Use as required and refer to the Manufacturer's expiry date.'
	6.3	2M Hydrochloric Acid changed to 6M.
	12.0	Section added to record Revision History.