Originating Department	QC
Approval Departments	QC, Validation
Approval Date	Refer to Q-Pulse
Effective Date	Refer to Q-Pulse

1.0 PRODUCT DETAILS

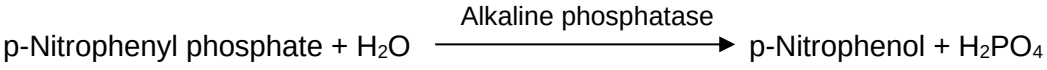
1.1 **Enzyme Name**: **Alkaline phosphatase**

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 400nm per unit time is a measure of the alkaline phosphatase activity[1].

3.0 UNIT DEFINITION

That amount of enzyme causing the hydrolysis of one micromole of p-Nitrophenyl phosphate per minute at pH 9.6 and 25˚C.

4.0 EQUIPMENT REQUIRED

Double beam UV/vis spectrophotometer with chart recorder
Water bath set to achieve a reaction temperature of 25˚C (± 0.1˚C)
Thermometer
Silica and plastic cuvettes
Test tubes
Manual pipettes and tips
0.2µm filter

5.0 REAGENTS REQUIRED

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

Reagent details

Chemical / Reagent	Supplier	Product No.	F.W.
Glycerol	VWR	24388.320	92.09
Glycine	Sigma	G7126	75.07
2M Sodium hydroxide	Fisher Scientific	71474-1L	N/A
Zinc chloride	VWR	29156.231	136.28
Magnesium chloride (hexahydrate)	Sigma	M2670	203.3
Phosphatase substrate	Sigma	P4744	371.14

6.0 PREPARATION OF REAGENTS

6.1 2M Sodium hydroxide

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

6.2 0.025M Glycine/NaOH pH 9.6

Dissolve 3.75g of glycine in approximately 1.9L of water and adjust the pH to 9.6 at 25˚C with 2M Sodium hydroxide. Adjust to a final volume of 2L with water.
Stable for 1 week at 2 to 8˚C

6.3 1M Glycine/NaOH pH9.6 stock buffer

Stock buffer preparation can be used as an alternative way to prepare 0.025M Glycine/NaOH pH 9.6

Dissolve 37.5g of glycine in approximately 450ml of water and adjust the pH to 9.6 at 25˚C with 2M Sodium hydroxide. Adjust to a final volume of 500ml with water. Filter via a 0.2µm filter into a suitably sized new container.
Stable at ambient temperature for 1 year

6.4 0.025M Glycine/NaOH pH9.6 prepared from 1M stock

Take 25ml of 1M Glycine/NaOH pH9.6 and make up to 1L with water. Stable for one week at 2 to 8˚C

6.5 1M Magnesium chloride

Dissolve 4.07g of Magnesium chloride (hexahydrate) in water and adjust to a final volume of 20ml.
Stable for 1 month at 2 to 8˚C

6.6 0.1M Zinc chloride

Dissolve 272mg of Zinc chloride in water and adjust to a final volume of 20ml.
Stable for 1 month at 2 to 8˚C

6.7 Enzyme diluent (0.025M Glycine/NaOH pH 9.6 / 1mM $MgCl_2$ / 0.1mM $ZnCl_2$ / 10% glycerol)

Add 1.0ml of 1M Magnesium chloride and 1.0ml of 0.1M Zinc chloride to 1L of 0.025M Glycine/NaOH pH 9.6. Add 100ml of glycerol and stir well. Readjust the pH if necessary to pH 9.6 at 25˚C with 2M Sodium hydroxide.
Stable for 2 weeks at 2 to 8˚C

6.8 0.0039M Phosphatase substrate

Dissolve the contents of one vial of phosphatase substrate (approx. 1g) in approximately 600ml of 0.025M Glycine/NaOH pH 9.6. Readjust to pH 9.6 at 25˚C with 2M Sodium hydroxide. Make up to a final volume that gives a phosphatase substrate concentration of 1.45mg/ml in 0.025M Glycine/NaOH pH 9.6. Store in a dark bottle.
Stable for 6 weeks at 2 to 8˚C

6.9 Working Substrate

Add together 120ml of 0.0039M phosphatase substrate, 1.25ml of 1M Magnesium chloride and approximately 15ml of water. Adjust to pH 9.6 at 25˚C with 2M Sodium hydroxide. Make up to 145ml with water, recheck the pH at 25˚C and readjust if necessary. Store in a dark bottle. Stable for 3 days at 2 to 8˚C. Recheck the pH at 25˚C each day and adjust to pH9.6 with 2M Sodium hydroxide. Discard if the pH is <9.50 or if the solution is very yellow.

6.10 Enzyme Solutions

Freeze-dried powders:

Into new glass vials accurately weigh at least 10mg of freeze-dried powder, each test sample to be weighed in triplicate. Dissolve each to a concentration of 5mg/ml in enzyme diluent. Immediately prior to assay, dilute to approximately 0.08 U/ml enzyme diluent.

Liquid preparations:
Immediately prior to assay, dilute to approximately 0.08 U/ml in enzyme diluent.

Process samples:
Dilute to approximately 0.08 U/ml in enzyme diluent, ensuring the concentration is within the range of 0.0165 U/ml to 0.165 U/ml (equivalent to reaction rates (ΔA_{400}/min) of 0.01 to 0.09)[1].

[1] Taken from Method Validation Report (MVR203)

7.0 TEST PROCEDURE

Temperature = 25˚C Wavelength = 400nm Light path = 10mm

Pipette the following into disposable, capped test tubes at 25˚C:

	TEST	REF
Working substrate	2.90ml	2.90ml
Enzyme diluent	0.00ml	0.10ml

Cap the test tubes and allow to equilibrate for approx. 5 minutes then add:

Enzyme solution	0.10ml	0.00ml
Total reaction mix volume (Vt) =	3.00ml	3.00ml

Mix and transfer to disposable cuvettes and record the increase in absorbance at 400nm, reading the test solution versus the reference solution for approximately 3 minutes. Measure the change in absorbance at 400nm per minute (ΔA_{400}nm/min) over the linear portion of the curve and use this value in the calculation.

8.0 CALCULATION

8.1 Volume activity (U/ml) = $\dfrac{\Delta A_{400}/\text{min} \times V_t \times \text{dilution factor}}{V_s \times \varepsilon}$

Where: V_t = final volume of the reaction mix (3.00ml)
V_s = sample volume (0.10ml)
ε = micromolar extinction coefficient for p-Nitrophenol (18.7 $cm^2/\mu mole$)

Volume activity (U/ml) = ΔA_{400}/min x 1.6 x dilution factor

8.2. For freeze-dried samples: Weight activity (U/mg material) = $\dfrac{\text{U/ml}}{\text{mg/ml}}$

$$\text{Specific activity (U/mg protein)} = \frac{\text{U/mg material}}{\text{mg protein/mg material}}$$

8.3 For liquid preparations:

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

9.0 PROTEIN DETERMINATION

Protein concentration is determined by the Biuret method in accordance with Analytical Procedure AP99

10.0 $A_{280}^{1\%}$ DETERMINATION

This is determined in accordance with Analytical Procedure AP63

11.0 ASSOCIATED DOCUMENTS

MST056	Alkaline Phosphatase Codes - ALPI12G, ALPIXG & ALPI Blending
MVR203	Validation of Analysis Procedure for Alkaline Phosphatase Activity
AP99	Biuret Protein Determination
AP63	Spectrophotometric Measurements

12.0 REFERENCES

Bergmeyer, H. U. (1974) *Methods of Enzymatic Analysis,* 2nd edition, p496, Academic Press, New York

13.0 REVISION HISTORY

Document Issue Number	Section Number	Summary of Changes
09	Global	Originating department now QC. Changed Header to reflect current practice; changed approval and effective date to 'Refer to Q-Pulse'. Changes for document version 08 removed; corrected grammatical and spelling errors.
	4.0	Added plastic cuvettes to the equipment list
	5.0	Reagent Details- Updated supplier and product numbers for Sodium hydroxide
	6.10	Added a section on dilution of process samples to include the range determined from the MBR.
	11.0	Changed Lowry protein to Biuret protein; added MVP203 & MST056