Originating Department	QC
Approval Departments	QA, QC & Validation
Effective Date	Refer to Q-pulse

1.0 PRODUCT DETAILS

1.1 **Enzyme Name**: Glucose Oxidase

1.2 **Systematic Name**: β–D-Glucose : oxygen 1-oxidoreductase

1.3 **E.C. Number**: 1.1.3.4

1.4 **Source**: Aspergillus niger

2.0 ASSAY PRINCIPLE

The procedure for the analysis of glucose oxidase is a modified method based on the method of Trinder[1].

Glucose oxidase catalyses the oxidation of glucose as shown below:

$$\beta\text{-D-glucose} + O_2 + H_2O \xrightarrow{\text{glucose oxidase}} \text{D-glucono-1,5-lactone} + H_2O_2$$

The amount of H_2O_2 produced is detected via a linked assay in the presence of peroxidase as shown below:

$$H_2O_2 + \text{4AAP} + \text{Phenol} \xrightarrow{\text{peroxidase}} H_2O + \text{Quinoneimine dye}$$

The formation of Quinoneimine dye can be measured spectrophotometrically at 500nm

3.0 UNIT DEFINITION

That amount of enzyme causing the oxidation of one micromole of glucose per minute at 25ºC and pH 7.0

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
Pipettes and tips

5.0 REAGENTS REQUIRED

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

Reagent details

Chemical / Reagent	Supplier	Product No.	F.W.
Di-potassium hydrogen phosphate	VWR	26931.263	174.18
Potassium dihydrogen phosphate	VWR	26936.293	136.09
B-D-Glucose	Sigma	G8270	180.16
4-Aminoantipyrine	Sigma/Aldrich	A4382	203.24
Phenol	VWR	20599.231	94.11
Peroxidase	BBI Solutions	HRP3C	N/A

6.0 PREPARATION OF REAGENTS

6.1 0.1M potassium phosphate pH 7.0

Combine 60mL of 1M dipotassium hydrogen phosphate with 40mL of 1M potassium dihydrogen phosphate and make to a final volume of 1L with water.

Stable for 2 weeks at 2 to 8°C.

6.2 17.2mM 4-Aminoantipyrine

Accurately weigh approximately 50mg of 4-Aminoantipyrine into a new glass vial and dissolve to a concentration of 3.5mg/ml in water. Store in a dark bottle.

Stable for 1 month at 2ºC to 8ºC.

6.3 159mM Phenol

Accurately weigh approximately 250mg of Phenol into a new glass vial and dissolve to a concentration of 15mg/ml in water. Store in a dark bottle.

Stable for 1 month at 2ºC to 8ºC.

6.4 1.39M β-D-glucose solution.

Accurately weigh 25g of β-D-glucose into a brand-new graduated container and add water to just below the 100mL graduation. Shake until completely dissolved and make up to 100mL with water. Allow to stand at room temperature for at least one hour to mutarotate prior to use.

Stable for 1 month stored at 2ºC to 8ºC.

6.5 Peroxidase solution (200 pyrogallol U/ml)

Weigh at least 10mg of HRP3C material into a new vial and dissolve to a concentration of 200 pyrogallol U/ml in 0.1M potassium phosphate pH 7.0.

Stable at 2 to 8°C for 2 weeks.

6.6 Enzyme solution.

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 0.1M potassium phosphate pH 7.0. Immediately prior to assay, dilute to approximately 0.47 U/ml in 0.1M potassium phosphate pH 7.0.

Liquid preparations/process samples:

Immediately prior to assay, dilute to approximately 0.47 U/ml in 0.1M potassium phosphate pH 7.0, ensuring the concentration is within the range 0.104 to 0.725 U/ml (equivalent to reaction rates (ΔA500/min) of 0.0104 to 0.0746)[1].

For Glucose Oxidase code GOL7 final product samples:

Immediately prior to assay, dilute to approximately 0.47 U/ml in 0.1M potassium phosphate pH 7.0, ensuring the concentration is within the range 0.332 to 0.622 U/ml (equivalent to reaction rates (ΔA500/min) of 0.0350 to 0.0622)[2].

7.0 TEST PROCEDURE

Temperature = 25°C. Wavelength = 500nm Light path = 10mm

Into disposable test tubes pipette the following:

	Test	**Reference**
0.1M potassium phosphate, pH 7.0:	2.2mL	2.3mL
1.39M β-D-glucose:	0.5mL	0.5mL
17.2mM 4 Aminoantipyrine	0.1mL	0.1mL
159mM Phenol	0.1mL	0.1mL
Peroxidase solution	0.1mL	0.1mL

Allow the solutions to equilibrate to 25°C for approximately 5 minutes, then add:

Enzyme solution, diluted to ~0.47U/ml:	0.10ml	0.00ml
Total volume (V_t):	3.10ml	3.10ml

[1] Derived from Analytical Method Validation ATMV04
[2] Derived from Analytical Method Validation ATMV06

Transfer to disposable cuvettes and place cuvettes in a spectrophotometer. Record the increase in absorbance at 500nm, reading the test solution against the reference solution for approximately 3 minutes. Measure the change in absorbance per minute (ΔA_{500}/min) over the linear portion of the curve and use this value in the calculation.

8.0 CALCULATION

Note: For consistency purposes, the glucose oxidase activity units determined via this analytical procedure will be converted to activity units derived via the original procedure, AP24, (using o-Dianisidine as hydrogen donor) by multiplying by a factor of 2.07. The 2.07 conversion factor will be embedded in all associated Macro Spreadsheet Templates. Converted values will be used for 'In Process' and 'Final Product' testing and hence appear on all associated documentation. The factor of 2.07 was derived from a Quality Control Study Report (QCSR038).

8.1 Volume activity (U/ml) = $\frac{\Delta A_{500}/\text{min} \times V_t \times 2.07 \times \text{dilution factor}}{V_s \times \varepsilon}$

Where: V_t = final volume of the reaction mix (3.10ml)
V_s = sample volume (0.10ml)
ε = micromolar extinction coefficient for quinoneimine dye (cm^2/micromole) = 6.8
2.07 is the correction factor applied to convert units to original procedure (AP24)

Volume activity (U/ml) = ΔA_{500}/min x 9.44 x dilution factor

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

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

8.3 For liquid samples: Specific activity (U/mg protein) = $\frac{\text{U/ml}}{\text{mg protein/ml}}$

9.0 PROTEIN DETERMINATION

Protein is determined by the method of Lowry et al in accordance with Analytical Procedure AP62 [2].

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

This is determined in accordance with Analytical Procedure AP63.

11.0 ASSOCIATED DOCUMENTS

AP62	Lowry Protein Determination
AP63	Spectrophotometric Measurements
MST058	Macro Spreadsheet Template for Glucose Oxidase Code GO3A
MST059	Macro Spreadsheet Template for Glucose Oxidase Code GO3B2

MST069 Macro Spreadsheet Template for Glucose Oxidase Code GO3B3
QCSR038 Investigation into an alternative Glucose Oxidase analytical procedure based on Phenol and 4-Aminoantipyrine

12.0 REFERENCES

1. Trinder, P. (1969) Ann. Clin. Biochem. **6**, 24

2. Lowry, O.H., Rosebrough, N.J., Farr, A.L., & Randall, R.J. (1951) *J. Biol. Chem*. **193**, 265

13.0 REVISION HISTORY

Document version number	Section number	Summary of Changes
01	N/A	New document
02	6.6	Validated dilution range concentrations for GOL7 final product material added from Analytical Test Method Validation, ATMV06