Originating Department	QC
Approval Departments	QA, QC & Validation
Approval Date	24th April 2018
Effective Date	2nd May 2018

1.0 PRODUCT DETAILS

1.1 **Enzyme Name**: Glucose Dehydrogenase (FAD-dependent)

1.2 **Systematic Name**: D-Glucose (flavin adenine dinucleotide) dehydrogenase

1.3 **E.C. Number**: 1.1.99.10

1.4 **Source**: Microbial

2.0 ASSAY PRINCIPLE

The reduction of 2,6-Dichlorophenol-indophenol (DCPIP) is measured at 600nm by spectrophotometry.

$$\beta\text{-D-Glucose} + \text{PMS} \xrightarrow{\text{FAD-Glucose Dehydrogenase}} \text{D-Glucono-1,5-lactone} + \text{PMS (reduced)}$$

$$\text{PMS (reduced)} + \text{DCPIP} \longrightarrow \text{PMS} + \text{DCPIP (reduced)}$$

3.0 UNIT DEFINITION

The amount of enzyme that catalyses the reduction of one micromole of 2,6-Dichlorophenol indophenol per minute at 37°C and pH 6.5.

4.0 EQUIPMENT REQUIRED

Double beam UV/vis spectrophotometer with chart recorder
Water bath set to achieve a reaction temperature of 37°C (±0.1°C)
Thermometer
Cuvettes
Test tubes
Manual pipettes and tips

5.0 REAGENTS REQUIRED

When using hazardous chemicals, handle in accordance with COSHH Regulations.

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
Bovine serum albumin (BSA)	Roche Diagnostics	10 735	N/A
D-(+)-Glucose	Sigma	G8270	180.16
2,6- Dichlorophenol-indophenol (DCPIP)	Sigma	D1878	290.08
Phenazine methosulfate (PMS)	Sigma	P9625	306.34

6.0 PREPARATION OF REAGENTS

6.1 0.05M potassium phosphate pH 6.5

Dissolve 3.40g of Potassium dihydrogen phosphate in water and adjust to a final volume of 500ml.

Dissolve 2.18g of di-Potassium hydrogen phosphate in water and adjust to a final volume of 250ml.

Titrate the Potassium dihydrogen phosphate with the di-Potassium hydrogen phosphate to obtain a pH of 6.5.

Stable for 2 weeks at 2 to 8°C.

6.2 Enzyme Diluent Buffer

Add 500mg of BSA to 500ml of 0.05M Potassium phosphate buffer. Allow 10 minutes for the BSA to soak into the buffer and then gently stir to avoid foaming.

Stable for 2 weeks at 2 to 8°C.

6.3 1M D-Glucose

Weigh 3.60g of D-(+)-Glucose into a new glass vial. Add approximately 12ml of water and shake vigorously until dissolved. Adjust to a final volume of 20ml with water. Shake well to homogenise. Allow to stand at room temperature for at least one hour to mutarotate before using.

Stable for 1 week at 2 to 8°C.

6.4 2mM 2,6-Dichlorophenol-indophenol solution (DCPIP)
Note: this is based on the anhydrous form, see F.W. in reagents table

Weigh approximately 10mg of DCPIP into a new glass vial and dissolve to a concentration of 0.58g/ml in water. Store in a dark bottle.

Stable for 5 days at 2 to 8°C.

6.5 15mM Phenazine methosulfate solution (PMS)

Weigh approximately 15mg into a new glass vial and dissolve to a concentration of 4.6mg/ml in water. Store in a dark bottle.

Stable for 5 days at 2 to 8°C.

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 0.05M potassium phosphate pH 6.5. Immediately prior to assay, dilute to approximately 0.12 – 0.22 U/ml in diluent buffer.

7.0 TEST PROCEDURE

Temperature = 37°C Wavelength = 600nm Light path = 10mm

Into disposable test tubes pipette the following:

0.05M Potassium phosphate pH 6.5	2.05ml
1M D-Glucose	0.60ml

Allow the solutions to equilibrate to 37°C for approximately 5 minutes , then add:

2mM DCPIP	0.15ml
15mM PMS	0.10ml
Enzyme solution, diluted to ~0.12 – 0.22 U/ml	0.10ml
Total Volume (Vt)	3.00ml

Mix and transfer to a disposable cuvette and measure the decrease in absorbance at 600nm for approx. 5 mins. versus air. Calculate the change in absorbance per minute (ΔA_{600}/min) over the linear portion of the trace.

N.B. Determine the blank rate (ΔA_{600}/min) by substituting the enzyme solution with enzyme diluent buffer in the procedure above. This blank rate must be subtracted from the ΔA_{600}/min for each test sample.

8.0 CALCULATION

8.1 Volume activity (U/ml) = $\frac{\Delta A_{600}/min_{(test)} - \Delta A_{600}/min_{(blank)} \times V_t \times \text{dilution factor}}{V_s \times \varepsilon}$

Where: V_t = final volume of the reaction mix (ml) = 3.00
V_s = sample volume (ml) = 0.1
ε = micromolar extinction coefficient for DCPIP ($cm^2/\mu mole$) = 16.3

Volume activity (U/ml) = $\Delta A_{600}/min_{(Test)} - \Delta A_{600}/min_{(Blank)} \times 1.84 \times$ dilution factor

8.2 For freeze-dried samples:

$$\text{Weight activity (U/mg material)} = \frac{\text{U/ml}}{\text{mg/ml}}$$

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

9.0 PROTEIN DETERMINATION

Protein is determined by the method of Lowry et al in accordance with Analytical Procedure AP62[1].

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

12.0 REFERENCES

1. 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
03	Global	Reformat throughout
	1.5	Section deleted to avoid unnecessary document amendments when product codes are introduced or obsoleted
	4.0	Equipment required amended to reflect current requirements
	5.0	Reagent details amended to reflect current suppliers
	6.1	Reference to 37°C removed because phosphate buffer is not sensitive to temperature Reference to analytical grade water removed Calculation amendment 2.20g changed to 2.18g (3 significant figures)
	6.2	0.5g changed to 500mgs
	6.3	Reference to weighing into new vial added to reflect current practice. Quantities changed to allow preparation of 20ml of reagent in a glass vial Allow to mutarotate highlighted in red type.
	6.4	Note added to ensure reagent prepared uses the anhydrous form of reagent Amount of DCPIP amended to 0.58mg/ml Into new glass vials added Reference to analytical grade water removed 'protect from light' amended to store in a dark bottle
	6.5	5-Methylphenazinium methyl sulphate now Phenazine methosulfate Into new glass vials added Reference to analytical grade water removed 'protect from light' amended to store in a dark bottle
	6.6	Statement into new glass vial added Instruction to weigh in triplicate added Liquid samples removed as only freeze-dried products are assayed
	7.0	Light path changed from 1cm to 10mm 'Diluted enzyme' changed to enzyme solution to match what it has previously been stated as 'in quick succession' removed
	8.0	Micromolar absorption coefficient amended to micromolar extinction coefficient to maintain consistency with other analytical procedures Reference to protein analysis removed and instead included in section 9.0
	9.0 and 10.0	Sections added to reference analytical procedures for protein and $A_{280}^{1\%}$ determination
	11.0	New section for Associated Documents added
	12.0	Section added for references