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

1.0 PRODUCT DETAILS

1.1 **Enzyme Name**: Mutarotase

1.2 **Systematic Name**: Aldose 1-epimerase

1.3 **E.C. Number**: 5.1.3.3

1.4 **Source**: Porcine kidney

2.0 ASSAY PRINCIPLE

Based on the method of Miwa and Okuda (1974)[1]

$$\alpha\text{-D-Glucose} \xrightarrow{\text{Mutarotase}} \beta\text{-D-Glucose}$$

$$\beta\text{-D-Glucose} + O_2 + H_2O \xrightarrow{\text{GO}} \text{D-Gluconolactone} + H_2O_2$$

The rate of oxygen consumption by the action of glucose oxidase in oxidising the β-D-Glucose is a measure of the mutarotase activity. The oxygen uptake is measured using a Rank oxygen electrode.

3.0 UNIT DEFINITION

That amount of enzyme causing an increase in the rate of spontaneous mutarotation of α-D-Glucose to β-D-Glucose of one micromole per minute at 25°C and pH 7.0

4.0 EQUIPMENT REQUIRED

Rank digital oxygen system with electrode with chart recorder
Water bath set to achieve a reaction temperature of 25°C (±0.1°C)
Double Beam UV/vis spectrophotometer with chart recorder
Thermometer
Disposable test tube
Pipettes and tips
Aerator
Silica Cuvettes

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.
2M Sodium Hydroxide	Fisher Scientific	71474-1L	N/A
α-D(+)-Glucose	Sigma-Aldrich	158968	180.16
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich	D8418	78.13
Ethylenediamine tetra acetic acid (EDTA)	Sigma-Aldrich	ED2SS	372.2
Glucose oxidase	BBI Solutions	GO3A	N/A

6.0 PREPARATION OF REAGENTS

6.1 2M Sodium hydroxide

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

6.2 0.02M Ethylenediamine tetra acetic acid (EDTA)

Dissolve 7.44g of EDTA in 800ml of water. Adjust the pH to 7.0 at 25°C with 2M Sodium hydroxide and make up to 1000ml with water. Aerate continuously at room temperature for use in assay substrate.

Stable for 1 week at 2 to 8°C.

6.3 α-D-Glucose (with β-D-Glucose contamination < 5%)

Weigh a minimum 50mg of α-D-Glucose into a clean glass vial and dissolve up to a concentration of 27mg/ml in dimethyl sulfoxide.

Prepare fresh daily.

6.4 Glucose oxidase solution

Use a BBI Solutions GO3A batch with concentration of approximately 6,500 U/ml.

e.g. for a GO3A of activity 260U/mg, dissolve to a concentration of 25mg/ml (6500 ÷ 260)

6.5 Enzyme solution

Freeze-dried powders:

Accurately weigh at least 10mg into new glass vials, each test sample to be weighed in triplicate. Dissolve to a concentration of 5mg/ml in 0.02M EDTA. Immediately prior to assay, dilute to approximately 12U/ml in 0.02M EDTA.

Liquid samples: Immediately prior to assay, dilute to approximately 12U/ml in 0.02M EDTA.

7.0 TEST PROCEDURE

Temperature = 25°C

Into the oxygen electrode chamber pipette the following:

	Test
Aerated EDTA solution	2.80ml
Glucose oxidase solution	0.03ml

Equilibrate for approximately 5 minutes whilst stirring at 25°C and set the chart recorder to 82.3 divisions. The chart recorder calibrated in accordance with AP158 (Loss of oxygen methods section) Allow to stabilise and adjust where necessary. Then add:

α-D-Glucose solution	0.03ml

Allow the rate to stabilise for 5 minutes as the β-D-Glucose is used up and the linear rate of spontaneous mutarotation is recorded. Start the reaction with:

Mutarotase sample	0.10ml

Record the new linear reaction rate over 2-3 minutes

Measure the enzyme-catalysed mutarotation rate over the linear portion of the curve in divisions per minute. Subtract the spontaneous mutarotation rate (division/min) taken immediately before the start of the reaction from the enzyme-catalysed mutarotation rate. Use this result in the calculation.

8.0 CALCULATION

$$\text{Volume activity (U/ml)} = \frac{(\Delta_2 - \Delta_1) \times 0.003125 \times 8.23 \times V_t \times \text{dilution factor}}{V_s}$$

$$= (\Delta_2 - \Delta_1) \times 0.762 \times \text{dilution factor}$$

Where:
Δ_1 = The rate of spontaneous mutarotation (divisions/min)
Δ_2 = The rate of enzyme-catalysed mutarotation (divisions/min)
0.003125 = The number of micromoles of glucose per division*
8.23 = The conversion factor from oxygen electrode units to polarimetric units

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

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

* The derivation is as follows:

From critical tables: air saturated water contains 8.23mg Oxygen per litre at 760mm of pressure and 25°C.
Now, with the recorder set to read 82.3 divisions:
82.3 divisions = 8.23mg oxygen per litre
Therefore, 1 division = 0.1mg Oxygen per litre
= 0.003125 micromoles of Oxygen per ml.

9.0 PROTEIN DETERMINATION

Protein is determined by the Biuret method in accordance with Analytical Procedure AP99.[2,3]

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

This is determined in accordance with Analytical Procedure AP63.

11.0 ASSOCIATED DOCUMENTS

AP99	Biuret Protein Determination
AP63	Spectrophotometric Measurements
AP158	Use of Oxygen Electrode

12.0 REFERENCES

1. Miwa and Okuda (1974), *J. Biol. Chem*. **75**, 1177-1179

2. Bergmeyer, H. (1974) Methods of Enzymatic Analysis. 2nd ed. Weinheim: Verlag Chemie, pp. 174 - 176.

3. Beisenherz G., Boltze H. J., Bücher Th., Czok R., Garbade K.H., Meyer-Arendt E.& Pfleiderer G. (1953) *Zeitshcr. f. Naturf*. **8b**, 555 (1953).

13.0 REVISION HISTORY

Document Issue Number	Section Number	Summary of Changes
06	Global	Header changed; approval/effective date changed to refer to Q-pulse
	4.0	Equipment list updated.
	5.0	Product codes and suppliers changed. Chemical safe usage statement altered to reference manufacturer's instructions.
	6.3	Minimum weighing of α-D-Glucose changed
	6.4	Product code changed and example added.
	9.0	Method of protein analysis altered from Lowry protein AP62 to Biuret protein AP99.
	11.0	Reference to Lowry protein AP removed and replaced with Biuret protein AP.
	12.0	Lowry protein references removed and replaced with Biuret protein references.