Originating Department	QA
Approval Departments	QA, QC & Validation
Approval Date	29th May 2018
Effective Date	31st May 2018

1.0 PRODUCT DETAILS

1.1 Enzyme Name: Superoxide dismutase

1.2 Systematic Name: Superoxide: superoxide oxidoreductase

1.3 E.C. Number: 1.15.1.1

1.4 Source: Bovine erythrocytes

2.0 ASSAY PRINCIPLE[2]

$$\text{Xanthine} + O_2 \xrightarrow{\text{Xanthine oxidase}} \text{Uric acid} + O_2^{\cdot -}$$

$$O_2^{\cdot -} + \text{Cytochrome C} \longrightarrow \text{Reduced cytochrome C}$$

$$O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ \xrightarrow{\text{SOD}} O_2 + H_2O_2$$

Superoxide competes with the Cytochrome C for superoxide ions produced in the aerobic Xanthine oxidase system, thus inhibiting the rate of reduction of Cytochrome C. The rate of change in the optical density at 550nm is a measure of the Superoxide dismutase activity.

3.0 UNIT DEFINITION

That amount of enzyme causing a 50% inhibition in the rate of reduction of Cytochrome C under the conditions of assay.

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
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.
2M Sodium hydroxide	Sigma	71474	N/A
Di-potassium hydrogen phosphate	VWR	26931.263	174.18
Potassium dihydrogen phosphate	VWR	26936.293	136.09
Ethylenediamine tetra-acetic acid di-sodium salt (EDTA)	Sigma	ED2SS	372.2
Cytochrome C	MP Biomedical	101467	12000
Xanthine sodium salt	Sigma	X2502	174.09
Xanthine oxidase	BBI Solutions	XO2	N/A

6.0 PREPARATION OF REAGENTS

6.1 0.05M Potassium phosphate/0.5mM EDTA, pH 7.8

Dissolve 3.40g of potassium di-hydrogen phosphate and 93.1mg EDTA in water and adjust to a final volume of 500ml. Reserve 50 ml for the preparation of 1.3mM Xanthine.

Dissolve 4.35g of di-potassium hydrogen phosphate and 93.1mg EDTA in water and adjust to a final volume of 500ml.

Titrate the 0.05M di-potassium hydrogen phosphate/0.5mM EDTA with the 0.05M potassium di-hydrogen phosphate/0.5mM EDTA to obtain a pH of 7.8 at 25°C.
Stable for 2 weeks at 2 to 8°C.

6.2 0.3mM Cytochrome C

Weigh approximately 35mg of Cytochrome C and dissolve to a concentration of 3.6mg/ml in 0.05M Potassium Phosphate 0.5mM EDTA, pH 7.8. Store in a dark bottle.
Stable for 5 days at 2 to 8°C.

6.3 2M Sodium hydroxide

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

6.4 1.3mM Xanthine

Dissolve 22.8mg of Xanthine in 35ml of water and add 3 drops of 2M Sodium hydroxide. Stir for 10 minutes. Add 60ml of 0.05M Potassium Phosphate 0.5mM EDTA, pH 7.8. Adjust the pH to 7.8 at 25°C with 0.05M potassium di-hydrogen phosphate, 0.5mM EDTA (held from section 6.1). Adjust to a final volume of 100ml with 0.05M Potassium Phosphate 0.5mM EDTA, pH 7.8.

6.5 Xanthine oxidase

Dilute the enzyme using 0.05M Potassium Phosphate 0.5mM EDTA, pH 7.8 to obtain a ΔA_{550}nm/min of 0.025 (when used in part 7.1 of the test procedure).
Prepare fresh daily.

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 0.5mM EDTA, pH 7.8. Immediately prior to assay, dilute to obtain a ΔA_{550}nm/min of 0.025 in 0.05M Potassium Phosphate 0.5mM EDTA, pH 7.8. (See section 7.1)

Liquid preparations:

Immediately prior to assay, dilute to obtain a ΔA_{550}nm/min of 0.025 in 0.05M Potassium Phosphate 0.5mM EDTA, pH 7.8. (See section 7.1).

7.0 TEST PROCEDURE

Temperature = 25°C Wavelength = 550nm Light path = 10mm

7.1 Xanthine oxidase activity measurement:

Into disposable test tubes pipette the following:

	Test	**Reference**
0.05M KPO_4, 0.5mM EDTA, pH 7.8	2.70ml	2.80ml
0.3mM Cytochrome C	0.10ml	0.10ml
1.3mM Xanthine	0.10ml	0.10ml

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

Xanthine oxidase	0.10ml	0.00ml
Total volume (V_t):	3.00ml	3.00ml

Mix, then transfer the solutions into disposable cuvettes and record the increase in absorbance at 550nm, reading the test solution against the reference solution for approximately 5 minutes. Measure the change in absorbance per minute over the linear portion of the curve. Repeat if necessary, changing the dilution of the Xanthine Oxidase until a ΔA_{550}nm/min of 0.025 is obtained. Use this dilution in the enzyme assay below.

7.2 Superoxide dismutase activity measurement:

Into disposable test tubes pipette the following:

	Test	Reference
0.05M KPO_4, 0.5mM EDTA, pH 7.8	2.60ml	2.80ml
0.3mM Cytochrome C	0.10ml	0.10ml
1.3mM Xanthine	0.10ml	0.10ml
Enzyme solution	0.10ml	0.00ml

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

Xanthine Oxidase ΔA_{550}/min of 0.025	0.10ml	0.00ml
Total volume (V_t):	3.00ml	3.00ml

Mix, then transfer the solutions into disposable cuvettes and record the change in absorbance at 550nm, reading the test solution against the reference solution for approximately 5 minutes. Measure the change in absorbance per minute over the linear portion of the curve. Repeat if necessary, changing the dilution of the Enzyme solution until a ΔA_{550}/min of 0.0125 is obtained. Use this dilution factor in the calculation.

8.0 CALCULATION

8.1 Volume activity (U/ml) = $\frac{D}{V_s}$

Where: D = dilution to obtain a ΔA_{550}/min of 0.0125
V_s = Sample volume (0.1ml)

8.2 For freeze-dried samples:

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

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

8.3 For liquid samples:

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

9.0 PROTEIN DETERMINATION

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

2. J.M. McCord and I. Fridovich, J. Biol. Chem. (1969), **244**, 6049

13.0 REVISION HISTORY

Document Issue Number	Section Number	Summary of Changes
04	1.5	Section removed to avoid the inclusion of specific codes which could lead to unnecessary revision updates as new products are introduced and/or codes are rationalised
	4.0	Equipment required amended to reflect current requirements
	5.0	Regent details amended to reflect current suppliers, reagents now tabular
	6.1	Reference to analytical grade water removed Calculation amendment to correct 5.70g to 4.35g
	6.2	Calculation amendment to instruct to dissolve up to 3.6mg/ml 36mg changed to approximately 35mg
	6.4	Reference to analytical grade water removed Reference to 'approximately' 35ml removed, 35ml is required 'to which has been added' amended to 'add' Instruction to adjust to a final volume of 100ml added to reflect current practice
	6.5	ΔE_{550}/min changed to ΔA_{550}/min to reflect current nomenclature Stable for four hours amended to prepare fresh daily to reflect current practice, reference to storage temperature removed because reagent is not stored, it would be discarded at the end of the day and prepared fresh the following day
	6.6	Section split into freeze-dried powders and liquid preparations Unused vials changed to new glass vials Approximately 20mg changed to at least 10mg Reference to storing on ice and using within one hour removed to reflect current practice Change in absorbance amended to increase in absorbance ΔE_{550}/min changed to ΔA_{550}/min to reflect current nomenclature
	7.0	Light path amended to 10mm
	7.1	Heading changed to 'Xanthine Oxidase activity measurement' 'plastic' test tubes amended to 'disposable' test tubes, 'at 25°C' removed, for approximately 5 mins added Total volume added ΔE_{550}/min changed to ΔA_{550}/min to reflect current nomenclature
	7.2	'plastic' test tubes amended to 'disposable' test tubes, 'at 25°C' removed, for approximately 5 mins added Total volume added ΔE_{550}/min changed to ΔA_{550}/min to reflect current nomenclature
	8.0	Split into 3 sections
	11.0	Section added to include associated documents, section 11.0 and 12.0 now 12.0 and 13.0