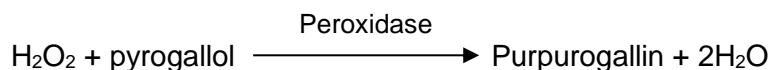


Originating Department	QC
Approval Departments	QA, QC & Validation
Approval Date	4 th August 2017
Effective Date	18 th August 2017

1.0 PRODUCT DETAILS

- 1.1 **Enzyme Name:** Peroxidase
- 1.2 **Systematic Name:** Donor: Hydrogen peroxide oxidoreductase
- 1.3 **E.C. Number:** 1.11.17
- 1.4 **Source:** Horseradish

2.0 ASSAY PRINCIPLE



The formation of Purpurogallin is measured spectrophotometrically at 420nm¹

3.0 UNIT DEFINITION

That amount of enzyme which catalyses the production of one milligram of Purpurogallin in 20 seconds at 20°C and pH 6.0

4.0 EQUIPMENT REQUIRED

Double beam UV/vis spectrophotometer with chart recorder
Water bath set to achieve a reaction temperature of 20°C (± 0.1°C)
Thermometer
Silica 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
Potassium dihydrogen phosphate	VWR	26936.293	136.09
Hydrogen peroxide solution (30%)	Merck	107209	34.01
Pyrogallol	Acros Organics	164561000	126.11

6.0 PREPARATION OF REAGENTS**6.1 2M Sodium hydroxide**

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

6.2 0.1M Potassium phosphate pH 6.0

Dissolve 6.80g of Potassium di-hydrogen phosphate in water and adjust to pH 6.0 at 20°C with 2M Sodium hydroxide. Make up to a final volume of 500ml with water.
Stable for 2 weeks at 2 to 8°C.

6.3 5.33% Pyrogallol

Dissolve 1.333g of Pyrogallol in water and adjust to a final volume of 25ml using a volumetric flask. Store in a dark bottle and prepare fresh daily.

6.4 Hydrogen peroxide solution

Dilute 0.66ml of Hydrogen peroxide in water and adjust to a final volume of 50ml using a volumetric flask. Store in a dark bottle and prepare fresh daily.

6.5 Enzyme solution

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 6.0. Immediately prior to assay, dilute to approximately 0.75 U/ml in 0.1M potassium phosphate pH 6.0.

7.0 TEST PROCEDURE

Temperature = 20°C Wavelength = 420nm Light path = 10mm

Into disposable test-tubes pipette the following:

	TEST	REF
0.1M potassium phosphate pH 6.0	2.40ml	2.50ml
5.33% pyrogallol	0.30ml	0.30ml
Hydrogen peroxide solution	0.20ml	0.20ml

Allow the solutions to equilibrate to 20°C for approximately 5 minutes, then add:

Enzyme solution (diluted to 0.75U/ml)	<u>0.10ml</u>	<u>0.00ml</u>
Total reaction mix volume (V _t)	<u>3.00ml</u>	<u>3.00ml</u>

Mix then record the increase in absorbance at 420nm, reading the test solution versus the reference solution for approximately 1 minute. Measure the change in absorbance per 20 seconds ($\Delta A_{420}/20\text{sec}$) over the linear portion of the curve and use this value in the calculation.

8.0 CALCULATION

$$\text{Volume activity (U/ml)} = \frac{\Delta A_{420}/20\text{sec} \times V_t \times \text{dilution factor}}{V_s \times 12}$$

Where: V_t = final volume of the reaction mix (ml) = 3.0
V_s = sample volume (ml) = 0.10
12 = absorbance of 1mg/ml solution of purpurogallin at 420nm

$$\text{Volume activity (U/ml)} = \Delta A_{420}/20\text{sec} \times 2.5 \times \text{dilution factor}$$

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

9.0 A₂₈₀^{1%} AND RZ DETERMINATION

This is determined in accordance with Analytical Procedure AP63.

10.0 ASSOCIATED DOCUMENTS

AP63 Spectrophotometric Measurements

11.0 REFERENCES

1. Modification of the method of Sumner, J. B. and Gjessing, E.C., (1943) *Biochem.* **2**, 1291

12.0 REVISION HISTORY

Document version number	Section number	Summary of Changes
04	Global	Reformat throughout
	1.5	Section removed
	4.0	Equipment required amended to reflect current requirements
	5.0	Reagent details amended to reflect current suppliers
	6.2	Reference to analytical grade water removed 5°C changed to 2 – 8°C Stability for 1 week adjusted to 2 weeks to reflect current practice
	6.3 and 6.4	Reference to analytical grade water removed Reference to storing on ice removed to reflect current practice
	6.5	New glass vials added Minimum weight of 10mg added Reference to storing on ice removed to reflect current practice
	7.0	Light path changed from 1cm to 10mm
	9.0	Section deleted and instead document AP63 referenced, combined with reference to A ₂₈₀ ^{1%} Determination
	10.0	New section for Associated Documents added