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

1.0 PRODUCT DETAILS

1.1 **Enzyme Name**: Catalase

1.2 **Systematic Name**: Hydrogen-peroxide:hydrogen-peroxide oxidoreductase

1.3 **E.C. Number**: 1.11.1.6

1.4 **Source**: Bovine liver & *Aspergillus niger*

2.0 ASSAY PRINCIPLE

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

The decrease in absorbance at 240nm is proportional to the catalase activity.

3.0 UNIT DEFINITION

That amount of enzyme causing the decomposition of one micromole of hydrogen peroxide per minute at 25°C 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 cuvettes
Test tubes
Manual 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.
6M Hydrochloric acid	Fisher Scientific	72033-1L	N/A
Di-sodium hydrogen phosphate dihydrate	VWR	28029.260	177.99
Potassium dihydrogen phosphate	VWR	26936.293	136.09
Hydrogen peroxide solution	Sigma	1.07209.0250	N/A

6.0 PREPARATION OF REAGENTS

6.1 6M Hydrochloric acid

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

6.2 0.05M Sodium potassium phosphate pH7.0

Dissolve 5.87g of di-sodium hydrogen phosphate dihydrate and 2.28g of potassium dihydrogen phosphate in approximately 900ml of water. Adjust to pH 7.0 with 6M Hydrochloric acid and make up to 1 litre with water.
Stable for 2 weeks at 2 to 8°C

6.3 Working substrate

Add approximately 0.64ml[1] of 30% hydrogen peroxide to 100ml of 0.05M Sodium potassium phosphate pH 7.0.

Pipette 2ml of 0.05M Sodium potassium phosphate pH 7.0 and 1ml of working substrate into a test tube. Mix thoroughly and transfer to a silica cuvette. Measure the absorbance at 240nm versus 0.05M Sodium potassium phosphate pH 7.0. If necessary, add more 0.05M Sodium potassium phosphate pH 7.0 or 30% hydrogen peroxide to the working substrate to adjust the concentration until and absorbance of 0.85 (±0.02) is obtained.

Store in a dark bottle. Stable for 1 month when stored at 2 to 8°C.

6.4 Enzyme solution

Freeze-dried powders:

Accurately weigh at least 10mg of freeze-dried powder into new glass vials, each test sample to be weighed in triplicate. Dissolve each to a concentration of 5mg/ml in 0.05M Sodium potassium phosphate pH 7.0. Immediately prior to assay, dilute to approximately 0.6U/ml in 0.05M Sodium potassium phosphate pH 7.0, the final 4.5ml serial dilution buffer having been pre-equilibrated at 25°C.[2]

[1] 0.64ml is a guide as it can vary between different lots and over time.
[2] Pre-equilibration is required since the volume of diluted enzyme (2ml) is a significant proportion of the reaction mixture volume (3ml) and hence will influence the reaction temperature.

Liquid preparations:

Immediately prior to assay, dilute to approximately 0.6U/ml in 0.05M Sodium potassium phosphate pH 7.0, the final 4.5ml serial dilution buffer having been pre-equilibrated at 25°C.[2]

Process samples:

Dilute to approximately 0.6U/ml in 0.05M Sodium potassium phosphate pH 7.0, ensuring the concentration is within the range of 0.0297 U/ml to 0.914 U/ml (equivalent to reaction rates (ΔA_{240}/min) of 0.0086 to 0.0266).[3]

7.0 PROCEDURE

Allow 0.05M Sodium potassium phosphate pH 7.0 and working substrate to equilibrate at 25°C for at least 5 minutes in the water bath before use.

Temperature = 25°C Wavelength = 240nm Light path = 10mm

Into disposable test tubes pipette the following:

	Test	**Reference**
Working substrate	1.00ml	0.00ml
0.05M Sodium potassium phosphate pH 7.0	0.00ml	3.00ml

Allow the solutions to equilibrate to 25°C then add:

Enzyme solution, diluted to ~0.6U/ml:	2.00ml	0.00ml
Total reaction mix volume (V_t)	3.00ml	3.00ml

Transfer the solutions to silica cuvettes then record the decrease in absorbance at 240nm, reading the test solution versus the reference solution for approximately 3 minutes. Measure the change in absorbance per minute (ΔA_{240}/min) over the linear portion of the curve and use this value in the calculation.

When measuring the rate of change for CAT2F product follow the instructions on the Catalase (Kinetic Assay) Addendum[4].

[3] Taken from Analytical Test Method Validation ATMV-013

[4] AP11a - Catalase (Kinetic Assay) Addendum

8.0 CALCULATION

8.1 Volume activity (U/ml) = $\frac{\Delta A_{240}/\text{min} \times V_t \times \text{dilution factor}}{V_s \times \varepsilon}$

Where: V_t = final volume of the reaction mix (ml) = 3.00
V_s = sample volume (ml) = 2.00
ε = micromolar extinction coefficient for peroxide ($cm^2/\mu mole$) = 0.0436

Volume activity (U/ml) = ΔA_{240}/min x 34.4 x dilution factor

8.2. For freeze-dried samples: 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 preparations:

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

9.0 PROTEIN DETERMINATION

Protein is determined by the method of Lowry et al in accordance with Analytical Procedure AP62[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
ATMV-013	Validation of Catalase (Kinetic Method)
AP11a	Catalase (Kinetic Assay) Addendum

12.0 REFERENCES

1. Valle, B. L. and Hoch F. L. (1955) *Proc. Nat. Acad. Sci.* **41**, 327

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

REVISION HISTORY

Document version number	Section number	Summary of Changes
09	Global	Header Changed to reflect current practice. Approval date removed and Effective Date changed to 'refer to Q-Pulse'. Removed document history summary for 08 to provide space for further changes.
	5.0	Reagent details- changed suppliers to Sigma and corrected product numbers from hydrochloric acid and hydrogen peroxide.
	7.0	Changed Enzyme solution, diluted to ~0.6U/ml from ~0.2U/ml in line with enzyme solution dilution in section 6.4.
10	Global	Header changed to version 10; QA added to the review panel; minor layout changes to ease reading of the document.
	5.0	Replaced; When using hazardous chemicals, handle in accordance with COSHH Regulations. With; When using the following reagents, refer to the manufacturer's instructions for safe handling and disposal.
	7.0	Section added to reference the new addendum
	11	Reference to Addendum added