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

1.0 PRODUCT DETAILS

1.1 **Enzyme Name:** Lipoxidase

1.2 **Systematic Name:** Linoleate : oxygen oxidoreductase

1.3 **E.C. Number:** 1.13.11.12

1.4 **Source:** Soybean

2.0 ASSAY PRINCIPLE

The procedure for the analysis of lipoxidase is based on the method of Bergmeyer.[1]

$$\text{Linoleate + oxygen} \xrightarrow{\text{Lipoxidase}} \text{13 hydroperoxyoctadeca-9, 11-dienoate}$$

The increase in absorbance at 234nm is a measure of the lipoxygenase activity.

3.0 UNIT DEFINITION

That amount of enzyme causing an increase in extinction at 234nm of 0.001 per minute at pH 9.0 and 25°C and pH 9.0.

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 hazardous chemicals, handle in accordance with COSHH Regulations.

Reagent details

Chemical / Reagent	Supplier	Product No.	F.W.
Sodium hydroxide	VWR	28244.262	40.0
Linoleic acid (Manufactured by Acros)	Fisher Scientific	215040050	280.45
Ethanol	Brenntag	DRS0000NH	46.07
Orthoboric acid	VWR	20185.260	61.83
Tween 80	Sigma	P1754	N/A

6.0 REAGENT PREPARATION

6.1 10M Sodium hydroxide

Dissolve 40.0g in approximately 60ml of water.
Caution: ***This solution may become very hot.***
Carefully adjust to a final volume of 100ml with water.
Stable for 1 month at ambient temperature (but discard if any particles are observed in the solution).

6.2 0.2M Borate/NaOH pH 9.0

Dissolve 12.4g of Orthoboric acid in approximately 800ml of water. Adjust the pH to 9.0 (±0.01) with 10M Sodium hydroxide at 25°C. Adjust to a final volume of 1L with water. Make up the day before required and store overnight at 2°C to 8°C ready for use the following day.
Stable for 5 days at 2°C to 8°C.

6.3 Substrate solution – Stock solution

6.3.1 Prepare a 0.04% solution of Tween 80 by weighing 40 ±2 mg of Tween 80 and make up to 100ml with water. Stir until completely dissolved. Cool at 2°C to 8°C for a minimum of 45 minutes.

6.3.2 Cool approximately 10ml of ethanol at 2°C to 8°C before use. Into a new glass vial pipette 8ml of ethanol. Add 0.05ml of linoleic acid and allow to dissolve at 2°C to 8°C. Do not stir to dissolve.
Note: Linoleic acid oxidises easily therefore is sensitive to light and air

6.3.3 Once dissolved, place the solution onto a magnetic stirrer and stir for 5 minutes.

6.3.4 While stirring, carefully add approximately 10ml of pre-cooled 0.04% Tween 80. The appearance of the solution will turn from clear to white. Allow to stand at 2°C to 8°C for 15 minutes.

6.3.5 Transfer the solution into a pre-cooled 50ml volumetric flask via a small funnel and, rinsing out the vial thoroughly with pre-cooled 0.04% Tween 80. Adjust to a final volume of 50ml with pre-cooled 0.04% Tween 80 solution.
Note: It is important to use labware that has been pre-cooled at 2°C to 8°C to prevent warming the solution

6.3.6 Store in a dark bottle which has been pre-cooled before use. Stable for 2 to 3 days at 2°C to 8°C.
Note: Store at 2°C to 8°C between use to prevent oxidation

6.4 Substrate solution – working solution

6.4.1 Using a P5000 pipette transfer 16.67mls of stock solution into a 100ml volumetric flask.

6.4.2 Adjust to a final volume of 100ml with pre-cooled 0.2M Borate buffer and mix thoroughly.

6.4.3 Store in a pre-cooled dark bottle and prepare fresh daily.
Note: Store at 2°C to 8°C between use to prevent oxidation

6.5 Enzyme solution

Freeze-dried powders:

Freeze-dried final product material must be exposed to two freeze thaw cycles prior to testing[1].
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 0.2M Borate/NaOH pH 9.0. Immediately prior to assay, dilute to approximately 9000 U/ml in 0.2M Borate/NaOH pH 9.0.

Liquid preparations:

Immediately prior to assay, dilute to approximately 9000 U/ml in 0.2M Borate/NaOH pH 9.0.

7.0 TEST PROCEDURE

Temperature = 25°C Wavelength = 234nm Light path = 10mm

Into disposable test tubes pipette the following:

	Test	**Reference**
0.2M Borate/NaOH pH 9.0:	0.95ml	1.00ml
Working substrate:	2.00ml	2.00ml

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

Enzyme solution, diluted to ~0.2U/ml:	0.05ml	0.05ml
Total volume (V_t):	3.00ml	3.00ml

Mix and transfer to silica cuvettes and record the increase in absorbance at 234nm, reading the test solution versus the reference solution for approximately 5 minutes. Measure the change in absorbance at 234nm per minute (ΔA_{234}nm/min) over the linear portion of the curve and use this value in the calculation.

[1] See HQ-QSR056 for details

8.0 CALCULATION

8.1 Volume activity (U/ml) = $\frac{\Delta A_{234}/\text{min} \times \text{dilution factor}}{V_s \times 0.001}$

Where: V_s = sample volume (0.05ml)
0.001 = See unit definition (section 3.0)

Volume activity (U/ml) = ΔA_{234}/min x 20,000 x dilution factor

8.2 For freeze-dried samples: Weight activity (U/mg material) = $\frac{\text{U/ml}}{\text{mg/ml}}$

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

8.3 For liquid samples: 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 ASSOCIATED DOCUMENTS

AP62	Lowry Protein Determination
ATMV015	Analytical Test Method Validation for Lipoxidase
QSR056	Stability Report for Lipoxidase LPX1

11.0 REFERENCES

1. Bergymeyer, H.U., Gawehn, K., & Grassl, M., (1974) *Methods in Enzymatic Analysis*. 2nd edn (Bergmeyer, H.U., ed) Vol 1, p483 – 484, Academic Press, New York.

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

12.0 REVISION HISTORY

Document version number	Section number	Summary of Changes
06	Global	Header changed; approval date removed & effective date changed to refer to Q-pulse; changes for version 05 removed.
	6.5	Spacings resolved, 'Freeze-dried final product material must be exposed to two freeze thaw cycles prior to testing' added due to findings from QSR056. Nothing in the testing process has changed
	6.5 & 7.0	Cooled & ice cold removed – not part of current practice
	10.0	Associated documents added