Originating Department	R&D
Approval Departments	QA, QC
Effective Date	20th August 2015

1.0 PRODUCT DETAILS

1.1 **Name:** Esterase EC 3.1.1.1

1.2 **Systematic Name:** Carboxy-ester hydrolase

1.3 **E.C. Number:** 232-773-7

1.4 **Source:** Porcine Liver

1.5 **Suitable for BBI Solutions code:** PLE-1F

2.0 ASSAY PRINCIPLE

$$\text{Ethyl Butyrate} + H_2O \xrightarrow{\text{Esterase}} \text{Butyric Acid} + \text{Ethanol}$$

The Butyric acid formed by the hydrolysis reaction is titrated with alkali to maintain a pH of 8.0. The amount of alkali added per unit time is a measure of the Esterase activity.

3.0 UNIT DEFINITION

That amount of enzyme which hydrolyses 1.0 µmole of ethyl butyrate to butyric acid and ethanol per minute at 25°C and pH 8.0.

4.0 EQUIPMENT REQUIRED

Water incubated vessel at 25°C (connected to a heated water bath)
Magnetic stirrer with magnetic follower
pH Meter
Calibrated stopwatch
UV Spectrophotometer
Automatic pipettes and tips
Silica and disposable cuvettes
Disposable test tubes
Appropriate glassware, bottles and measuring cylinders

5.0 RELATED DOCUMENTS

EOP6701 – Cecil UV Spectrophotometers
EOP6724 – pH Meters
EOP6730 – Operation of Automatic Pipettes and Dispensing Equipment

6.0 CHEMICALS / REAGENTS REQUIRED

When using the reagents please refer to manufacturer instructions for safe handling and disposal.

Chemical / Reagent	Supplier	Product No.	F.W.
Ortho-boric acid	VWR International	20185.260	61.83
Ethyl Butyrate (99% solution)	Sigma Aldrich	E15701	116.16
2M Sodium Hydroxide solution	Sigma Aldrich (Fluka)	71474	40.00
0.01M Sodium Hydroxide solution	Sigma Aldrich (Fluka)	38227	40.00

7.0 PREPARATION OF REAGENTS

7.1 10mM Borate Buffer, pH 8.0 at 25°C (Reagent 1)

Dissolve 0.62g of ortho-boric acid in approximately 800ml of analytical grade water and while stirring adjust pH to 8.0 at 25.0°C with 2M sodium hydroxide solution. Make up to 1 litre with analytical grade water and re-check the pH. This buffer may be stored at 2-8°C for 1 week.

7.2 0.1% (v/v) Ethyl Butyrate Solution (Reagent 2)

N.B. Preparation of 250ml as outlined below is sufficient for 10 assays, increase the amount prepared if more assays are to be performed.

Pipette 0.25ml of Ethyl Butyrate (99% solution) to approx. 240ml of Reagent 1 and then make up to 250ml with the same. Ensure the solution is mixed thoroughly and store at 2-8°C for up to 2 days.

7.3 Ethyl Butyrate, 99% Solution (Reagent 3)

No reagent preparation required.

N.B. Ensure that a small aliquot (~1ml) is removed from the stock container to an appropriately labelled vial for use on that day. This removes the risk of accidentally contaminating the stock solution by repeated use.

7.4 0.01M Sodium Hydroxide (Reagent 4)

Transfer 0.5ml of 2M Sodium Hydroxide solution to a 100ml volumetric flask and make to the 100ml mark with analytical grade water. Mix thoroughly by inversion. This reagent must be prepared fresh daily.

N.B. *Ensure that a small aliquot (~2.5ml) is transferred from the volumetric flask to an appropriately labelled test tube prior to each assay. This removes the risk of accidentally contaminating the stock solution by repeated pipetting.*

7.5 Enzyme Test Solution (Reagent 5)

Freeze-dried powders:
Dissolve at 10mg/ml in 10mM Borate Buffer, pH 8.0 (Reagent 1). Keep cold and immediately prior to assay dilute to approximately 50U/ml in the same buffer.

Liquid samples:
Immediately before use dilute to approximately 50U/ml in cold 10mM Borate Buffer, pH 8.0 (Reagent 1).

8.0 TESTING PROCEDURE

Using a suitable pH meter (calibrated pH 7 to pH 10 before use) in conjunction with a magnetic stirrer, pipette the following reagents into a suitable titration vessel at 25 ±0.2°C:

	TEST
Reagent 2 (0.1% Ethyl Butyrate solution)	**25.00ml**

Allow to equilibrate for approx. 5 minutes then add:

Reagent 3 (99% Ethyl Butyrate solution)	**0.025ml**

Adjust the pH to approx. 8.1 using Reagent 4 (0.01M NaOH) then add:

Reagent 5 (Enzyme Test Solution)	**0.100ml**

When the pH drops to 8.0 start a stopwatch. Run the reaction for 5 minutes maintaining the pH of the reaction mixture at 8.0 by the addition of 0.050ml aliquots of Reagent 4.

Record the total volume (ml) of Reagent 4 required to maintain the pH at 8.0 over the 5 minute period. Use this value in the calculation below.

9.0 CALCULATION

$$\text{Volume activity (U/ml)} = \frac{0.01 \times \text{NaOH Volume} \times 1000 \times \text{dilution factor}}{T \times V_s}$$

Where:

0.01	= Molarity of NaOH
NaOH Volume	= Total volume (ml) of Reagent 5 required to maintain pH of 8.0
1000	= Conversion factor from millimoles to micromoles
T	= 5 (Reaction time in minutes)
V_s	= 0.1 (Enzyme Test Sample Volume)

Volume activity (U/ml) = NaOH Volume x 20 x dilution factor

For freeze-dried powders:

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

10.0 A_{280} DETERMINATION

10.1 Determine the A_{280} of esterase samples by measuring the absorbance at 280nm using a UV spectrophotometer. Prior to measurement fill two silica cuvettes with Reagent 1 and zero the machine.

10.2 Samples with expected A_{280} values greater than 1.0 must be diluted in Reagent 1. The A_{280} value is calculated by multiplying the absorbance reading at 280nm by the dilution factor.

10.3 Units/A_{280} is calculated by dividing the esterase activity (U/ml) of a test sample by the A_{280} of that sample.

$$U/A_{280} = \frac{\text{Activity (U/ml)}}{A_{280}}$$

11.0 REVISION HISTORY

Document Issue Number	Section Number	Summary of Changes
1	Global	New Analytical procedure