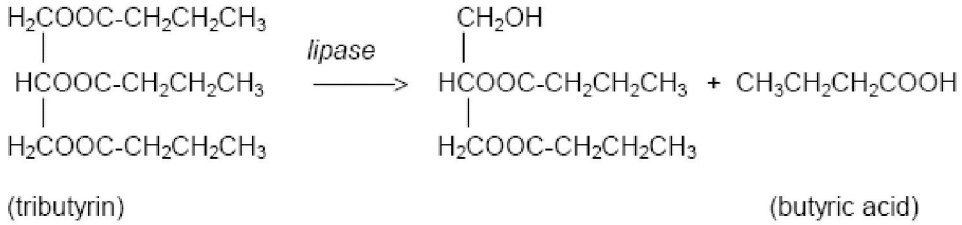
Lipase activity test – 1% tributyrin assay

METHOD:

This method describes the procedure to determine the activity of enzyme in tributyrin units per gram enzyme (TBU/g).

PRINCIPLE

The method is based on the speed at which the enzyme hydrolyzes tributyrin at pH 7.5.
The butyric acid which is formed is titrated with sodium hydroxide and the consumption of the latter recorded as a function of time.



(tributyrin) (butyric acid)

Analysis conditions:

Temperature:	40°C ± 1°C
pH:	7.5
Substrate:	tributyrin (glycerol tributyrate)
Reaction time:	at least 3 minutes, up to 30 minutes. (Only the linear response is used to calculate the slope.)

3. DEFINITION OF UNITS

1 TBU (lipase unit) is the amount of enzyme which releases 1 µmol titratable butyric acid per minute under the given standard conditions.

4. APPARATUS

pH-stat titration system, eg. Metrohm titrator, comprising:

- Metrohm 665 Dosimat
- Metrohm 614 Impulsomat
- Tamson waterbath
- pH electrode
- magnetic stirrer
- double wall vessel (100 ml)

4. REAGENTS AND SUBSTRATES
4.1 Chemicals

Sodium hydroxide (NaOH), (prepare 0.1 M solution), Sigma S5881
Glycerol tributyrate (tributyrin), e.g. Sigma-Aldrich 113026

Potassium dihydrogen phosphate p.a. (KH_2PO_4), (prepare 0.1 M solution) e.g. Sigma P2222

Buffer solution pH 7.0, e.g. Radiometer 943-112
Buffer solution pH 4.01, e.g. Radiometer 943-111

5. PROCEDURE

add to a 100 ml double walled reaction vessel (kept on 40 °C) equipped with a magnetic stirrer bar:

20 ml 25 mM Pi buffer pH 7.3 (= 5 ml 100 mM Pi buffer pH 7.3 + 15 ml demineralized water)
200 microliter tributyrin

stir for 2 minutes
switch on titrator (use 0.1 M sodium hydroxide)
adjust pH to 7.5

if a blanc reaction runs, monitor the base consumption per minute, for 3 minutes.

add enzyme (~30 units maximum). *Make sure to take a representative sample containing all particle sizes, specific activity may vary with particle size.*

wait until the first 100 µl base is added
reset and monitor the base consumption for 5 minutes, record each 0.5 minutes and calculate ml titrant / min

wash the vessel with ethanol, then water, dry with paper

6. CALCULATION

The measurements for the enzyme standards are used to plot a standard curve with enzyme activity as the x-axis and the associated mean slope (ml/min) as the y-axis. The data must be fitted to a straight line. The mean slope for the different dilutions of the samples is then used to read off the corresponding enzyme activity values from the standard curve. The activity of each sample is then calculated as follows:

$$\text{Sample activity (in TBU/g)} = \frac{\text{ml titrant/min} * \text{molarity of titrant} * 1000}{\text{sample weigth (g)}}$$

Unit definition

1 TBU unit = 1 µmol butyric acid released per minute / g enzyme