# Protease activity test - ethyl lactate assay

**METHOD:**

This method describes the procedure to determine the activity of enzyme in ethyl lactate units per gram enzyme (ELU/g).

**PRINCIPLE**

The method is based on the speed at which the enzyme hydrolyzes ethyl lactate (30% (v/v)) at pH 6.8. The lactic acid which is formed is titrated with sodium hydroxide and the consumption of the latter recorded as a function of time.

Analysis conditions:

| | |
|---|---|
| Temperature: | 25°C ± 1°C |
| pH: | 6.8 |
| Substrate: | ethyl lactate (L-lactic acid ethyl ester, ethyl-(S)-lactate) |
| Reaction time: | 5 minutes blanc followed by 5 minutes enzymatic hydrolysis |

**3. DEFINITION OF UNITS**

1 ELU (protease unit) is the amount of enzyme which releases 1 µmol titratable lactic 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 Carbonate (Na2CO3), (prepare 0.1 M solution), Sigma 223484
L-lactic acid ethyl ester, e.g. Sigma-Aldrich E34102
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

mix in a beaker:
5 ml 100 mM Pi buffer pH 6.5 and 9 ml demi water and
6 ml ethyl lactate
add this mixture to a 100 ml double walled reaction vessel (kept on 25 °C) equipped with a magnetic stirrer bar:

stir for 2 minutes
switch on titrator (use 0.1 M sodium hydroxide)
adjust pH to 6.8

monitor the base consumption for 5 minutes and record the total amount.
add enzyme (~30 units maximum).
wait until the first 100 µl base is added
reset and monitor the base consumption for 5 minutes, record each 0.5 minutes

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

*Make sure to take a representative sample containing all particle sizes, specific activity may vary with particle size.*

## 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:

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

## Unit definition

1 ELU unit = 1 µmol lactic acid released per minute / g enzyme