

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:	at least 3 minutes, up to 30 minutes. (Only the linear response is used to calculate the slope.)

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. (KH₂PO₄), (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 25 °C) equipped with a magnetic stirrer bar:

5 ml 100 mM Pi buffer pH 6.8 and 9 ml demi water

6 ml ethyl lactate

stir for 2 minutes

switch on titrator (use 0.1 M sodium hydroxide)

adjust pH to 6.8

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 3 - 30 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:

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

Unit definition

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