



酯酶

PRINCIPLE:

Ethyl Butyrate + H₂O (Esterase) > Butyric Acid + Ethanol

CONDITIONS: T = 25 °C, pH = 8.0

METHOD: Titrimetric

REAGENTS:

A. 10 mM Borate Buffer, pH 8.0 at 25 °C

(Prepare 500 ml in deionized water using Boric Acid. Adjust to pH 8.0 with 1 M NaOH.)

B. 0.1% (v/v) Ethyl Butyrate Solution

(Prepare 200 ml in Reagent A using Butyric Acid Ethyl Ester)

C. Ethyl Butyrate

(Use Butyric Acid Ethyl Ester)

D. 10 mM Sodium Hydroxide Solution-Standardized (NaOH)

(Prepare 50 ml in cold deionized water using Sodium Hydroxide, Pellets,. Standardize according to the ACS Reagent Procedure.)

E. Esterase Enzyme Solution

(Immediately before use, prepare a solution containing 50 units/ml of Esterase in cold Reagent A.)

PROCEDURE:

Using a suitable pH meter in conjunction with a magnetic stirrer, pipette (in milliliters) the following reagents into a suitable titration vessel:

	Tes
Reagent B (Ethyl Butyrate Soln)	25.0
Equilibrate to 25 °C. Then add:	
Reagent C (Ethyl Butyrate)	0.025



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Adjust the pH to 8.1 using Reagent D (NaOH). Then add:	
Reagent E (Enzyme Solution)	0.10

Start a timer when the pH reaches 8.0. Run the reaction for 1-5 minutes. Maintain the pH of the reaction mix at pH 8.0 by the addition of small volumes (0.050 ml) of Reagent D. Record the volume of Reagent D used to maintain the pH at 8.0 and the time required to consume the added Reagent D.

CALCULATION:

$$\text{Units/ml enzyme} = \frac{(\text{Molarity of NaOH})(\text{NaOH})(1000)(\text{df})}{(\text{T})(0.10)}$$

NaOH = Volume (in milliliters) of Reagent D used in the assay

1000 = Conversion factor from millimoles to micromoles (Unit definition)

df = Dilution factor

T = Time required (in minutes) to consume the added Reagent D (NaOH) while maintaining the pH at 8.0

0.1 = Volume (in milliliter) of enzyme used

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will hydrolyze 1.0 μ mole of ethyl butyrate to butyric acid and ethanol per minute at pH 8.0 at 25°C.

INITIAL ASSAY CONCENTRATIONS:

In a 25.125 ml reaction mix, the initial concentrations are 10 mM borate, 0.2% (v/v) ethyl butyrate and 5 units esterase.