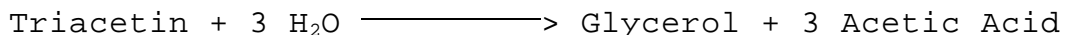


**Enzymatic Assay of ACETYLESTERASE
(EC 3.1.1.6)**

PRINCIPLE:



CONDITIONS: T = 30°C, pH 6.5

METHOD: Titrimetric

REAGENTS:

- A. 150 mM Sodium Chloride
(Prepare 200 ml in deionized water using Sodium Chloride, Sigma Prod. No. S-9625.)
- B. 20 mM Sodium Hydroxide Solution - Standardized (NaOH)
(Prepare 100 ml in deionized water using Sodium Hydroxide, 1.0 N, Sigma Stock No. 930-65.)
- C. 5% (v/v) Triacetin Solution, pH 6.5 at 30°C
(Prepare 100 ml in Reagent A using Triacetin (C2:0), Sigma Prod. No. T-5376. Adjust to pH 6.5 with 1 M NaOH.)
- D. Acetylcsterase Enzyme Solution
(Immediately before use, prepare a solution containing 20 units/ml of Acetylcsterase in cold deionized water.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent C (Triacetin)	20.0	20.0

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PROCEDURE: (continued)

Equilibrate to 30°C. Then add:

	<u>Test</u>	<u>Blank</u>
Reagent D (Enzyme Solution)	0.05	-----
Deionized Water	-----	0.05

Run the reaction for 5 minutes. Maintain the pH of the reaction mix at pH 6.5 by the addition of small volumes of Reagent B. Record the time required to add 0.050 ml Reagent B and maintain the pH at 6.5.

CALCULATION:

$$\text{units/ml enzyme} = \frac{(M)(\text{ml NaOH for Test} - \text{ml NaOH for Blank})(1000)(df)}{(\text{Time})(0.05)}$$

M = Molarity of NaOH solution

1000 = Conversion from millimoles to micromoles

df = Dilution factor

Time = Time required (in minutes) to maintain pH of 6.5

0.05 = 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 produce 1.0 μ mole of acetic acid from triacetin per minute at pH 6.5 at 30°C.

INITIAL ASSAY CONCENTRATION:

In a 20.05 ml reaction mix, the initial concentrations are 150 mM sodium chloride, 5% triacetin, and 4 units acetylcetase.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.