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SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of ESTERASE
(EC 3.1.1.1)
Sigma Prod. No. E-2884, E-3019, and E-3128

PRINCIPLE:

Ethyl Butyrate + H_2O $\xrightarrow{\text{Esterase}}$ 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, Sigma Prod. No. B-0252. 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, Sigma Prod. No. B-2391.)

C. Ethyl Butyrate
(Use Butyric Acid Ethyl Ester, Sigma Prod. No. B-2391.)

D. 10 mM Sodium Hydroxide Solution-Standardized (NaOH)
(Prepare 50 ml in cold deionized water using Sodium Hydroxide, Pellets, Sigma Prod. No. S-5881. Standardize according to the ACS Reagent Procedure.[1])

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:

	Test
Reagent B (Ethyl Butyrate Soln)	25.0

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PROCEDURE: (continued)

Equilibrate to 25°C. Then add:

	Test
Reagent C (Ethyl Butyrate)	0.025

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:

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

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 µmole 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.

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REFERENCES:

(1993) *Reagent Chemicals ACS Specifications*, 8th ed., p 95, American Chemical Society, Washington, DC

Adler, A.J. and Kistiakowsky, G.B. (1962) *Journal of the American Chemicals Society* **84**, 695-703

NOTES:

1. Standardization of NaOH is described in the (1993) *Reagent Chemical ACS Specification* reference.

2. This assay is based on the assay procedure described in Adler, A.J. and Kistiakowsky, G.B. (1962).

3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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