Enzymatic Assay of CHOLINESTERASE, BUTYRYL
(EC 3.1.1.8)

PRINCIPLE:

Butyrylcholine + H_2O $\xrightarrow{\text{Cholinesterase, Butyryl}}$ Butyrate + Choline

CONDITIONS: T = 37°C, pH = 8.0

METHOD: Titrimetric

REAGENTS:

A. 1600 mM Magnesium Chloride Solution
(Prepare 15 ml in deionized water using Magnesium Chloride, Hexahydrate, Prod. No. M-0250.)

B. 1000 mM Sodium Chloride Solution
(Prepare 50 ml in deionized water using Sodium Chloride, Prod. No. S-9625.)

C. 4 mM Butyrylcholine Chloride Solution (BuCholine)
(Prepare by dissolving 336 mg of Butyrylcholine Chloride, Prod. No. B-2753, in 350 ml deionized water. Then add
10 ml Reagent A, and 40 ml Reagent B for a total volume of 400 ml.)

D. 20 mM Sodium Hydroxide Solution-Standardized (NaOH)
(Prepare 50 ml in cold deionized water using Sodium Hydroxide, Stock No. 505-8. Standardize according to the ACS Reagent Procedure.[1])

E. Cholinesterase, Butyryl Enzyme Solution
(Immediately before use, prepare a solution containing 30 - 60 units/ml of Cholinesterase, Butyryl in cold deionized water.)

Enzymatic Assay of CHOLINESTERASE, BUTYRYL (EC 3.1.1.8)

PROCEDURE:

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

	Test
Reagent C (BuCholine)	50.00

Equilibrate to 37°C. Adjust to pH 8.5 with Reagent D (NaOH), using a burette. Then add:

Reagent E (Enzyme Solution)	0.40

Run the reaction for 1 - 5 minutes. Record the time when the pH reaches 8.0. Maintain the pH of the reaction mix at pH 8.0 by the addition of small volumes (0.05 ml) of Reagent D. Record the volume of Reagent D used to maintain the pH at 8.0 and the time required.

CALCULATION:

$$\text{Units/mg enzyme} = \frac{(\text{Molarity of NaOH})\ (\text{NaOH})\ (1000)}{(\text{T})\ (\text{mg enzyme/RM})}$$

NaOH = Volume (in milliliters) of Reagent D used in the assay
1000 = Conversion from millimoles to micromoles (Unit definition)
T = Time of assay
RM = Reaction Mix

UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of butyrylcholine to choline and butyrate per minute at pH 8.0 at 37°C.

INITIAL ASSAY CONCENTRATIONS:

In a 50.4 ml reaction mix, the initial concentrations are 40 mM magnesium chloride, 99 mM sodium chloride, 4 mM butyrylcholine chloride and 12 - 24 units cholinesterase, butyryl.

REFERENCE:

(1993) *Reagent Chemicals ACS Specifications*, 8th edition, 95.

Enzymatic Assay of CHOLINESTERASE, BUTYRYL (EC 3.1.1.8)

NOTES:

1. Standardization of the NaOH solution is described in the cited reference.

2. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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