SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of CHOLINESTERASE, ACETYL
(EC 3.1.1.7)
Sigma Prod. Nos. C-5021 and C-5400

PRINCIPLE:

Acetylcholine + H_2O $\xrightarrow{\text{Cholinesterase, Acetyl}}$ Choline + Acetic Acid

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, Sigma Prod. No. M-0250.)

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

C. 4 mM Acetylcholine Chloride Solution (Acetylcholine)
(Prepare 400 ml in 350 ml deionized water, 10 ml Reagent A, and 40 ml Reagent B using Acetylcholine Chloride, Sigma Prod. No. A-6625.)

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

E. Cholinesterase, Acetyl Enzyme Solution
(Immediately before use, prepare a solution containing 3-5 units/ml of Cholinestase, Acetyl in cold deionized water.)

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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 (Acetylcholine)	50.00

Equilibrate to 37°C. Adjust to pH 8.3 with Reagent D, using a burette. Then add:

Reagent E (Enzyme Solution)	0.4

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:

$$\text{Units/ml enzyme} = \frac{(\text{Molarity of NaOH})\ (\text{NaOH})\ (1000)\ (\text{df})}{(T)(0.4)}$$

NaOH = Volume (in milliliters) of Reagent D used in the assay
1000 = Conversion form millimoles to micromoles (Unit Definition)
df = Dilution factor
T = Time of assay (Unit Definition)
0.4 = Volume (in milliliter) of enzyme used

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

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

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UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of acetylcholine to choline and acetate 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, 100 mM sodium chloride, 4 mM acetylcholine chloride and 1.2 - 2 units cholinesterase acetyl.

REFERENCES:

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

NOTES:

1. Standardization of NaOH solution is described in the cited reference..
2. This assay is based on the cited reference.
3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.