上海源叶生物科技有限公司
Shanghai yuanye Bio-Technology Co., Ltd
电话：021-61312973　　传真：021-55068248
网址：www.shyuanye.com
邮箱：shyysw@sina.com

乙酰胆碱酯酶（电鳗鱼）

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.)

B. 1000 mM Sodium Chloride Solution

(Prepare 50 ml in deionized water using Sodium Chloride.)

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.)

D. 20 mM Sodium Hydroxide Solution-Standardized (NaOH)

(Prepare 50 ml in cold deionized water using Sodium Hydroxide.　)

E. Cholinesterase, Acetyl Enzyme Solution

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

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:

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

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

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

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

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.