Enzymatic Assay of PROTEASE INSOLUBLE

PRINCIPLE:

BAEE + H_2O $\xrightarrow{\text{Protease}}$ Na-Benzoyl-$_L$-Arginine + Ethanol

Abbreviation used:
BAEE = Na-Benzoyl-$_L$-Arginine Ethyl Ester

CONDITIONS: T = 30°C, pH 7.0

METHOD: Titrimetric

REAGENTS:

A. 1 M Potassium Chloride Solution (KCl)
(Prepare 15 ml in deionized water using Potassium Chloride, Sigma Prod. No. P-4504.)

B. 500 mM Na-Benzoyl-Arginine Ethyl Ester (BAEE)
(Prepare 15 ml in deionized water using Na-Benzoyl-$_L$-Arginine Ethyl Ester, Hydrochloride, Sigma Prod. No. B-4500. Adjust to pH 7.0 at 30°C with 1 M NaOH.)

C. 100 mM Sodium Hydroxide Titrant, Standardized (NaOH)
(Prepare 50 ml in deionized water using Sodium Hydroxide, Anhydrous, Sigma Stock No. 505-8. Standardize according to the ACS Reagent Procedure.[1])

D. Protease Insoluble Enzyme Solution (Insol Enz)
(Immediately before use, prepare a suspension containing approximately 20 mg of Protease, Insoluble Enzyme, 8 ml of deionized water and 1 ml of Reagent A. Mix by swirling. Filter on Whatman #50 filter paper and remove any soluble protease by vacuum filtration. Stop the vacuum filtration before the gel becomes dry or begins to crack. It should be moist.)

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PROCEDURE:

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

	Test
Deionized Water	8.00
Reagent A (KCl)	1.00
Reagent B (BAEE)	1.00

Mix by swirling and equilibrate to 30°C. Adjust to pH 7.0 by the addition of Reagent C (NaOH). Then add:

Reagent D (Insol Enz)	20 mg

Start a timer when the pH reaches 7.0. Run the reaction for 2 - 10 minutes. Maintain the pH of the reaction mix at
pH 7.0 by the addition of small volumes (0.05 ml) of Reagent C (NaOH). Record the volume of Reagent C (NaOH used to maintain the pH at 7.0) and the time (in minutes) required to consume the added Reagent C (NaOH).

CALCULATIONS:

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

NaOH = Volume (in microliters) of Reagent C (NaOH)
1000 = Conversion factor from mg to g
T = Time required (in minutes) to consume the added Reagent C
(NaOH) while maintaining the pH at 7.0

UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of BAEE per minute at pH 7.0 at 30°C.

FINAL ASSAY CONCENTRATIONS:

In a 10.00 ml reaction mix, the final concentrations are 100 mM potassium chloride, 50 mM Na-benzoyl-L-arginine ethyl ester, and 20 mg of protease insoluble enzyme.

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REFERENCE:

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

Arnon, R. (1970) *Methods in Enzymology*, XIX, 226-228

NOTES:

1. Standardization of NaOH is described in the (1993) Reagent Chemicals ACS Specifications.

2. This assay is based on the cited references.

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