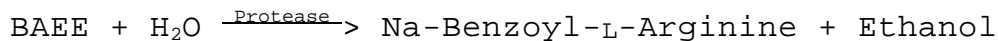


Enzymatic Assay of PROTEASE INSOLUBLE

PRINCIPLE:



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:

	<u>Test</u>
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
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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 μ mole 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.

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