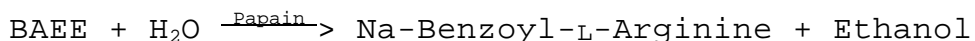


**Enzymatic Assay of PAPAINE, INSOLUBLE  
(EC 3.4.22.2)**

**PRINCIPLE:**



Abbreviation used:

BAEE = Na-Benzoyl-L-Arginine Ethyl Ester

**CONDITIONS:** T = 30°C, pH = 7.0

**METHOD:** Titrimetric

**REAGENTS:**

- A. 500 mM Potassium Chloride and 250 mM Na-Benzoyl-L-Arginine Ethyl Ester Solution (BAEE)  
(Prepare 20 ml in deionized water using Potassium Chloride, Sigma Prod. No. P-4504 and Na-Benzoyl-L-Arginine Ethyl Ester, Hydrochloride, Sigma Prod. No. B-4500.)
- B. 250 mM Ethylenediaminetetraacetic Acid Solution, pH 7.0 at 30°C (EDTA)  
(Prepare 100 ml in deionized water using Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Prod. No. ED2SS. Adjust to pH 7.0 at 30°C with 1 M NaOH.)
- C. 250 mM L-Cysteine Solution<sup>1</sup>, pH 7.0 at 30°C (L-Cys)  
(Prepare 20 ml in deionized water using L-Cysteine Hydrochloride, Monohydrate, Sigma Prod. No. C-7880. Adjust to pH 7.0 at 30°C with 1 M NaOH. **PREPARE FRESH.**)
- D. 100 mM Sodium Hydroxide Titrant, Standardized<sup>2</sup> (NaOH)  
(Prepare 50 ml in deionized water using Sodium Hydroxide, Anhydrous, Sigma Stock No. 505-8. Standardize according to the ACS Reagent Procedure.)

**Enzymatic Assay of PAPAIN, INSOLUBLE  
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**REAGENTS:** (continued)

- E. Papain Enzyme Suspension  
(Immediately before use, prepare a suspension containing  
2 - 4 mg/ml of Insoluble Enzyme attached to Beaded  
Agarose, Sigma Prod. No. P-4406, or 25 - 50 mg/ml of  
Papain, Insoluble Enzyme attached to Carboxymethyl  
Cellulose, Prod. No. P-8011, in cold deionized water.)

**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>
Reagent A (BAEE)	4.00
Reagent B (EDTA)	0.40
Reagent C (L-Cys)	4.00

Equilibrate to 30°C and adjust the pH to 7.0 by the addition of Reagent D (NaOH). Then add:

Reagent E (Enzyme Suspension) <sup>3</sup>	11.60
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Immediately mix by swirling. Readjust the pH to slightly above pH 7.0.

When the pH reaches 7.0, begin the timer. Maintain the pH of the reaction mix at 7.0 by the addition of small volumes (0.05 ml) of Reagent D (NaOH). Record the volume of

Reagent D (NaOH) used to maintain the pH at 7.0 and the time required for the consumption of Reagent D (NaOH). Run the reaction 1 - 5 minutes.

**CALCULATIONS:**

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

NaOH = Volume (in microliter) of NaOH used

1000 = Conversion factor from mg to g

T = Time (in minutes) required to consume the added  
Reagent D (NaOH) while maintaining the pH at 7.0

RM = Reaction Mix

**Enzymatic Assay of PAPAIN, INSOLUBLE  
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**UNIT DEFINITION:**

One unit will hydrolyze 1.0  $\mu$ mole of BAEE per minute at pH 7.0 at 30°C.

**FINAL ASSAY CONCENTRATIONS:**

In a 20 ml reaction mix, the final concentrations are 100 mM potassium chloride, 50 mM Na-benzoyl L-arginine ethyl ester, 5 mM ethylenediaminetetraacetic acid, 50 mM L-cysteine, 2 - 4 mg of insoluble papain attached to beaded agarose or 25 - 50 mg of insoluble papain attached to carboxymethyl cellulose.

**REFERENCE:**

(1993) *Reagents Chemicals ACS Specification*, 8th ed., 95, American Chemical Society, Washington DC

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

**NOTES:**

1. Adjust the pH quickly because L-cysteine readily oxidizes and forms a hazy solution.
2. Standardization of NaOH is described in (1993) *Reagents Chemicals ACS Specifications*.
3. The enzyme suspension should be mixed by swirling before addition so that it is homogeneous.
4. This assay is based on the cited references.
5. 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.**