

**Enzymatic Assay of PAPAINE/FICIN
On Agarose
(EC 3.4.22.2)**

PRINCIPLE:

BAEE ~~Papain/Ficin~~ → a-N-Benzoyl-L-Arginine + Ethanol

Abbreviations used:

BAEE = a-N-Benzoyl-L-Arginine Ethyl Ester

CONDITIONS: T = 25°C, pH = 7.0

METHOD: Titrimetric

REAGENTS:

- A. 500 mM Potassium Chloride Solution (KCl)
(Prepare 100 ml in deionized water using Potassium Phosphate, Prod. No. P-4504.)
- A. 250 mM Benzoyl-L-Arginine Ethyl Ester Solution (BAEE)
(Prepare 20 ml in Reagent A using a-N-Benzoyl-L-Arginine, Ethyl Ester, Prod. No. B-4500.)
- C. 250 mM Ethylenediaminetetraacetic Acid (EDTA)
(Prepare 100 ml in deionized water using Ethylenediaminetetraacetic Acid, Disodium, Dihydrate, Stock No. ED2SS.)
- D. 250 mM L-Cysteine, pH 7.0 at 25°C
(Prepare 25 ml in Reagent B using L-Cysteine Hydrochloride, Prod. No. C-7880. Adjust to pH 7.0 with 1 M NaOH.¹ **PREPARE FRESH.**)
- E. 100 mM Sodium Hydroxide Titrant, Standardized (NaOH)
(Prepare according to ACS Reagent Procedure)
- F. Papain/Ficin Enzyme Solution
(Immediately before use prepare a solution containing 2.5 mg/ml in cold deionized water. Allow to hydrate before use.)

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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>
Reagent A (BAEE)	4.0
Reagent C (EDTA)	0.4
Reagent D (L-Cysteine)	4.0

Equilibrate at 25°C, and adjust to pH 7.0 by the addition of Reagent E. then add:

Reagent F (Enzyme Solution)	11.6
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Monitor the pH of the reaction mix and record the time when the pH reaches 7.0. Add a known amount of Reagent E (NaOH) and record the time for the pH to return to 7.0. Repeat the process 3 times.

CALCULATION:

$$\text{Units/g Solid} = \frac{(\text{Vol NaOH consumed/min}) (\text{N NaOH}) (1000 \mu\text{Moles/mMole}) (1000)}{(\text{mg Sample/RM})}$$

N NaOH = Normality of Reagent E
1000 = Conversion of milligrams to grams (Unit definition)
RM = Reaction Mix (20 ml)

UNIT DEFINITION:

One unit will hydrolyze 1.0 μMole of BAEE per minute at pH 7.0 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 20 ml reaction mix the final concentrations are 100 mM potassium chloride, 50 mM BAEE, 5 mM EDTA, 50 mM L-cysteine and 25 mg papain/ficin agarose.

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NOTES:

1. Adjust the pH quickly because L-cysteine readily oxidizes and forms a hazy solution.
2. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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