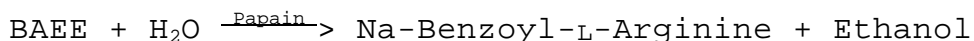


Enzymatic Assay of PAPAIN¹
(EC 3.4.22.2)

PRINCIPLE:



Abbreviation used:

BAEE = Na-Benzoyl-L-Arginine Ethyl Ester

CONDITIONS: T = 25°C, pH = 6.2

METHOD: Titrimetric Rate Determination

REAGENTS:

- A. 80 mM Na-Benzoyl-L-Arginine Ethyl Ester (BAEE)
(Prepare 50 ml in deionized water using Na-Benzoyl-L-Arginine Ethyl Ester Hydrochloride, Sigma Prod. No. B-4500.)
- B. 20 mM Ethylenediaminetetraacetic Acid (EDTA)
(Prepare 100 ml in deionized water using Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS.)
- C. 50 mM L-Cysteine, pH 6.2 at 25°C
(Prepare 25 ml in Reagent B using L-Cysteine Hydrochloride, Monohydrate, Sigma Prod. No. C-7880. Adjust to pH 6.2 with 1 M NaOH.² **PREPARE FRESH.**)
- D. 3 M Sodium Chloride Solution (NaCl)
(Prepare 25 ml in deionized water using Sodium Chloride, Sigma Prod. No. S-9625.)
- E. 20 mM Sodium Hydroxide Titrant, Standardized (NaOH)
(Prepare 50 ml in cold deionized water using Sodium Hydroxide, Anhydrous, Sigma Stock No. 505-8. Standardize according to the ACS Reagent Procedure.³)
- F. Papain Enzyme Solution
(Immediately before use, prepare a solution containing approximately 1 unit/ml in cold deionized water.)

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PROCEDURE:

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

	<u>Test</u>
Reagent A (BAEE)	7.0
Reagent C (L-Cysteine)	1.0
Reagent D (NaCl)	1.0

Equilibrate at 25°C, and adjust to pH 6.3 by the addition of Reagent E (NaOH). Then add:

Reagent F (Enzyme Solution)	1.0
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Monitor the pH of the reaction mix and record the time when the pH reaches 6.2. Maintain the pH of the reaction mix at pH 6.2 by the addition of small volumes of Reagent E and record the time (in minutes) when a total of 50 µl of Reagent E is consumed.⁴ Repeat this process for approximately 5 - 10 minutes.⁵ Plot the volume (ml) of 100 mM NaOH consumed vs the time (minutes) and determine the maximum linear rate from the plot. Use the time for the consumption of 50 µl of 100 mM NaOH in the calculation.

CALCULATION:

$$\text{Units/ml enzyme} = \frac{(0.05)(\text{Normality of NaOH})(1000)(df)}{(T)(1)}$$

0.05 = Volume (in milliliters) of Reagent E used to maintain

the pH at 6.2

1000 = Conversion from millimoles to micromoles

df = Dilution factor

T = Time (in minutes) required to maintain the pH at 6.2 per

50 µl aliquot

1 = Volume (in milliliter) of enzyme used

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

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

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UNIT DEFINITION:

One unit will hydrolyze 1.0 μ mole of BAEE per minute at pH 6.2 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 10 ml reaction mix the final concentrations are
56 mM Na-benzoyl-L-arginine ethyl ester,
2.0 mM ethylenediaminetetraacetic acid, 5.0 mM L-cysteine,
300 mM sodium chloride and 1.0 unit papain.

REFERENCE:

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

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

NOTES:

1. This assay is not to be used to assay Papain, Insoluble enzyme attached to beaded agarose, Sigma Prod. No. P-4406, and Papain, Insoluble enzyme attached to carboxymethyl cellulose, Sigma Prod. No. P-8011.
2. Adjust the pH quickly because L-cysteine readily oxidizes and forms a hazy solution.
3. Standardization of NaOH is described in (1993) Reagent Chemicals ACS Specifications.
4. Do not add the entire 50 μ l aliquot in one delivery. This will raise the pH to greater than pH 6.3 which may result in an erroneous rate.
5. There must be at least 4 additions of 50 μ l of NaOH during this time period. If there is not, increase the enzyme concentration and repeat.
6. This assay is based on the cited references.
7. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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