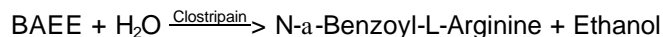


Enzymatic Assay of CLOSTRIPAIN (EC 3.4.22.8)

PRINCIPLE:



Abbreviations used:

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

CONDITIONS: T = 25°C, pH = 7.6, $A_{253\text{nm}}$, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 10 mM MOPS HCl Buffer with 2.5 mM Dithiothreitol and 1.0 mM Calcium Chloride, pH 7.4 at 25°C (Activation Soln)
(Prepare 100 ml in deionized water using MOPS, Sodium Salt, Sigma Prod. No. M-9381, DL-Dithiothreitol Sigma Prod. No. D-0632 and Calcium Chloride, Dihydrate, Sigma Prod. No. C-3881. Adjust the pH to 7.4 at 25°C with 1 M HCl.)
- B. 25 mM Sodium Phosphate Buffer with 2.5 mM Dithiothreitol and 0.25 mM N-a-Benzoyl-L-Arginine Ethyl Ester, pH 7.6 at 25°C (Assay Cocktail)
(Prepare 30 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751, DL-Dithiothreitol, Sigma Prod. No. D-0632, and N-a-Benzoyl-L-Arginine Ethyl Ester, Hydrochloride, Sigma Prod. No. B-4500. Adjust the pH to 7.6 at 25°C with 1 M NaOH.)
- C. Clostripain Enzyme Solution
(Approximately 2 - 3 hours before use, prepare a solution containing 0.5 - 1.5 unit/ml of Clostripain in Reagent A. Store at room temperature. The enzyme should be activated after this time period.)¹

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PROCEDURE:

Pipette (in milliliters) the following reagents into suitable quartz cuvettes:

	<u>Test</u>	<u>Blank</u>
Reagent B (Assay Cocktail)	3.00	3.00

Equilibrate to 25°C. Monitor the $A_{253\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent C (Enzyme Solution)	0.05	-----
Reagent A (Activation Soln)	-----	0.05

Immediately mix by inversion and record the increase in $A_{253\text{nm}}$ for approximately 5 minutes. Obtain the $r A_{253\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r A_{253\text{nm}}/\text{min Test} - r A_{253\text{nm}}/\text{min Blank}) (3.05) (df)}{(1.15) (0.05)}$$

3.05 = Total Volume (in milliliters) of assay

df = Dilution factor

1.15 = Millimolar extinction coefficient of N-a-Benzoyl-L-Arginine at 253 nm

0.05 = Volume (in milliliters) of enzyme used.

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

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

UNIT DEFINITION:

One unit will hydrolyze 1.0 μmole of BAEE per minute at pH 7.6 at 25°C in the presence of 2.5 mM DTT.

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FINAL ASSAY CONCENTRATIONS:

In a 3.05 ml reaction mix, the final concentrations are 25 mM sodium phosphate, 0.25 mM N- α -benzoyl-L-arginine ethyl ester, 0.02 mM calcium chloride, 0.16 mM MOPS, 2.5 mM dithiothreitol, and 0.025-0.075 unit clostripain.

REFERENCE:

Mitchell, W.M., and Harrington, W.F. (1968) *Journal of Biological Chemistry* **243**, 4683-4692.

NOTES:

1. The clostripain enzyme solution can also be activated by storing a 0.50-1.5 μ m/ml stock solution in Reagent A overnight at 5°C.
1. This assay is based on the cited reference.
2. 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.