

Enzymatic Assay of BROMELAIN (EC 3.4.22.4)

PRINCIPLE:

N α -CBZ-L-Lysine p-Nitrophenyl Ester $\xrightarrow{\text{Bromelain}}$ p-Nitrophenol + N α -CBZ-L-Lysine

Abbreviations:

CBZ = Carbobenzoxy

CONDITIONS: T = 25°C, pH = 4.6, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 30 mM Sodium Acetate Buffer with 100 mM Potassium Chloride and 1.0 mM L-Cysteine, pH 4.6 at 25°C
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625, L-Cysteine, Hydrochloride, Monohydrate, Sigma Prod. No. C-7880, and Potassium Chloride, Sigma Prod. No. P-4504. Adjust to pH 4.6 with 1 M HCl.)
- B. 50 mM N α -CBZ-L-Lysine p-Nitrophenyl Ester (LNPE)
(Prepare in 0.2 ml of Acetonitrile, Sigma Prod. No. A-3396, using N α -CBZ-L-Lysine P-Nitrophenyl Ester, Hydrochloride, Sigma Prod. No. C-3637. Dilute to 1.0 ml with deionized water. **PREPARE FRESH.**)¹
- C. Bromelain Enzyme Solution
(Prepare a solution containing 0.2 - 0.4 units/ml of Bromelain in Reagent A.)²

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.60	2.70
Reagent C (Enzyme Solution)	0.10	-----

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PROCEDURE: (continued)

Mix by inversion and equilibrate to 25°C. Then add:

	<u>Test</u>	<u>Blank</u>
Reagent B (LNPE)	0.10	0.10

Immediately mix by inversion and record the increase in $A_{340\text{nm}}$ for approximately 5 minutes. Obtain the $\Delta A_{340\text{nm}}$ /minute using the maximum linear rate for both the Tests and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340\text{nm}}/\text{min Test} - \Delta A_{340\text{nm}}/\text{min Blank})(2.8)(\text{df})}{(6.32)(0.1)}$$

2.8 = Volume (in milliliters) of assay

df = Dilution factor

6.32 = Millimolar extinction coefficient of p-Nitrophenol at 340 nm

0.1 = Volume (in milliliter) of enzyme used

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

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

UNIT DEFINITION:

One unit will release 1.0 μmole of p-nitrophenol from $\text{N}\alpha\text{-CBZ-L-lysine p-nitrophenyl ester}$ per minute at pH 4.6 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 2.80 ml reaction mix, the final concentrations are 29 mM sodium acetate, 96 mM potassium chloride, 0.96 mM $\alpha\text{-cysteine}$, 1.8 mM $\text{N}\alpha\text{-CBZ-L-lysine p-nitrophenyl ester}$, and 0.02 - 0.04 units bromelain.

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REFERENCES:

(1976) in *Methods in Enzymology*, **45**, 740-751

NOTES:

1. Solutions should be used as soon as possible since they hydrolyze on standing.
2. Allow enzyme solution to set on ice for approximately 2 hours.
3. This assay is based on the cited reference.
4. 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.