

## Enzymatic Assay of BROMELAIN INHIBITOR

### PRINCIPLE:

Bromelain Inhibitor will inhibit the following reaction:

LNPE  $\xrightarrow{\text{Bromelain}}$  Na-CBZ-L-Lysine + p-Nitrophenol

Abbreviations used:

LNPE = Na-CBZ-L-Lysine p-Nitrophenyl Ester

CBZ = Carbobenzoxy

**CONDITIONS:** T = 25°C, pH = 4.6,  $A_{317\text{nm}}$ , Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

### REAGENTS:

- A. 20 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, Potassium Chloride, Sigma Prod. No. P-4504, and L-Cysteine, Hydrochloride, Monohydrate, Sigma Prod. No. C-7880. Adjust to pH 4.6 at 25°C with 1 M HCl. **PREPARE FRESH.**)
- B. 90% (v/v) Acetonitrile  
(Prepare 1.0 ml in deionized water using Acetonitrile, Sigma Prod. No. A-3396.)
- C. 1.25 mM Na-CBZ-L-Lysine p-Nitrophenyl Ester Solution<sup>1</sup>  
(LNPE)  
(Prepare 1.0 ml in Reagent B using Na-CBZ-L-Lysine p-Nitrophenyl Ester, Hydrochloride, Sigma Prod. No. C-3637.)
- D. 10 mM Sodium Acetate with 100 mM Potassium Chloride, pH 4.6 at 25°C (Enz Dil)  
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No S-8625, and Potassium Chloride, Sigma Prod. No. P-4504. Adjust to pH 4.6 at 25°C with 1 M HCl.)

**Enzymatic Assay of BROMELAIN INHIBITOR**

**REAGENTS:** (continued)

- E. Bromelain Enzyme Solution<sup>2</sup> (Bromelain)  
(Immediately before use, prepare a solution containing 0.5 - 0.7 unit/ml of Bromelain, Sigma Prod. No. B-5144, in Reagent D.)
- F. Bromelain Inhibitor Solution<sup>3</sup> (Inhib)  
(Immediately before use, prepare a solution containing 0.14 mg/ml of Bromelain Inhibitor in Reagent D.)

**PROCEDURE:**

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

	<u>Uninh</u>	<u>Test1</u>	<u>Test2</u>	<u>Test3</u>	<u>Test4</u>	<u>Blank</u>
Reagent A (Buffer)	3.00	3.00	3.00	3.00	3.00	3.00
Reagent D (Enz Dil)	0.05	0.03	0.02	0.01	----	0.10
Reagent E (Bromelain)	0.05	0.05	0.05	0.05	0.05	----
Reagent F (Inhib)	----	0.02	0.03	0.04	0.05	----

Mix by inversion and equilibrate to 25°C. Allow the solution to incubate for a minimum of 2 minutes at 25°C. This is required for full activation of the bromelain. Monitor the A<sub>317nm</sub> until constant, using a suitably thermostatted spectrophotometer. Then add:

	<u>Uninh</u>	<u>Test1</u>	<u>Test2</u>	<u>Test3</u>	<u>Test4</u>	<u>Blank</u>
Reagent C (LNPE)	0.05	0.05	0.05	0.05	0.05	0.05

Immediately mix by inversion and record the increase in A<sub>317nm</sub> for approximately 5 minutes. Obtain the r A<sub>317nm</sub>/minute using the maximum linear rate for both the Tests and Blank.

**CALCULATIONS:**

$$\% \text{ Inhibition} = \frac{(r A_{317nm} \text{ Uninh} - r A_{317nm} \text{ Test}) (100)}{r A_{317nm} \text{ Uninh} - r A_{317nm} \text{ Blank}}$$

r A<sub>317nm</sub> Uninh = the uninhibited rate  
(Bromelain Inhibitor is not present)

Plot the Percent Inhibition versus the milligrams of Inhibitor in the reaction mix. Obtain the milligrams of Inhibitor at 50% inhibition from the graph.

## Enzymatic Assay of BROMELAIN INHIBITOR

### CALCULATIONS: (continued)

$$\text{mg Inhibitor/mg Bromelain} = \frac{(\text{mg Inhibitor})}{(\text{mg Bromelain/RM})}$$

mg Inhibitor = The milligrams of inhibitor at 50% inhibition  
(obtained from the graph)

RM = Reaction mix (Total volume = 3.15 ml)

### SPECIFICATIONS:

Approximately 0.6 mg will inhibit the activity of 1 mg purified bromelain (Sigma Prod. No. B-5144) by 50%.

### REFERENCES:

Heinrikson, R.L., and Kézdy, F.J. (1976) in *Methods in Enzymology* **45**, 740 - 751

### FINAL ASSAY CONCENTRATIONS:

In a 3.15 ml reaction mix, the final concentrations are 19 mM sodium acetate, 98 mM potassium chloride, 0.95 mM L-cysteine, 1.4% (v/v) acetonitrile, 0.02 mM Na-CBZ-L-lysine p-nitrophenyl ester, 0.025 - 0.035 unit bromelain, and 3 - 7 µg bromelain inhibitor.

### NOTES:

1. The concentration of the substrate solution **MUST** be precise. This solution is stable for several days when stored at 0 - 5°C.
2. Bromelain, Sigma Prod. No. B-5144, is used because other bromelain enzymes will give different results.
3. The concentration of the Bromelain Inhibitor solution **MUST** be precise.
4. Bromelain Unit Definition: One unit will release 1.0 µmole of p-nitrophenol from Na-CBZ-L-lysine p-nitrophenyl ester per minute at pH 4.6 at 25°C.
5. This assay is based on the cited reference.

- 6 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.**