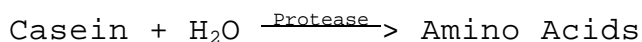


## Enzymatic Assay of PROTEASE

### PRINCIPLE:



**CONDITIONS:** T = 30°C, pH = 11.0,  $A_{275\text{nm}}$ , Light path = 1 cm

**METHOD:** Spectrophotometric Stop Rate Determination

### REAGENTS:

- A. 100 mM Sodium Tetraborate Solution  
(Prepare 10 ml in deionized water using Borax, Sigma Prod. No. B-9876.)
- B. 0.6% (w/v) Casein with 10.0 mM Sodium Tetraborate, pH 11.0 at 30°C (Casein).  
(Prepare by dissolving 600 mg of Casein, Sigma Prod. No. C-7078 in 4 ml of 0.1 M NaOH in a hot water bath (60°C). Cool to room temperature and then add 10 ml of Reagent A and 80 ml of deionized water. Adjust to pH 11.0 at 30°C with 1 M NaOH and bring to a volume of 100 ml with deionized water.)
- C. 110 mM Trichloroacetic Acid with 220 mM Sodium Acetate and 330 mM Acetic Acid (TCA)  
(Prepare 40 ml in deionized water using Trichloroacetic Acid, 6.1 N, approximately 100% (w/v), Sigma Stock No. 490-10, Sodium Acetate Trihydrate, Sigma Prod. No. S-8625, and Acetic Acid, Glacial, Sigma Prod. No. A-6283.)
- D. 10.0 mM Sodium Tetraborate Buffer, pH 11.0 at 30°C (Enz Dil I)  
(Prepare 10 ml in deionized water using Borax, Sigma Prod. No. B-9876.)
- E. 2.0 mM Calcium Acetate Solution (Enz Dil II)  
(Prepare 10 ml in deionized water using Calcium Acetate, Sigma Prod. No. C-1000.)

## Enzymatic Assay of PROTEASE

### REAGENTS:

- F. Protease Enzyme Solution  
(Prepare by dissolving 1 mg of Protease in 2 ml of ice-cold Reagent D. Immediately before use, dilute to 0.20 - 0.30 unit/ml of Protease with Reagent E.)

### PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent B (Casein)	3.00	3.00

Equilibrate to 30°C. Then add:

Reagent F (Enzyme Solution)	0.50	-----
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Immediately mix by swirling and incubate at 30°C for exactly 10 minutes. Then add:

Reagent C (TCA)	3.20	3.20
Reagent F (Enzyme Solution)	-----	0.50

Mix by swirling and incubate at 37°C for 20 minutes. Filter the solutions through Whatman #50 filter paper and transfer the solutions to suitable cuvettes and record the  $A_{275\text{nm}}$  for both the Test and Blank using a suitable spectrophotometer.

### CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{275\text{nm}} \text{ Test} - A_{275\text{nm}} \text{ Blank})(6.7)(\text{df})}{(10)(1.34)(0.5)}$$

6.7 = Total volume (in milliliters) of assay

df = Dilution factor

10 = Time (in minutes) of the assay per the Unit

Definition

1.34 = Millimolar extinction coefficient<sup>1</sup> of tyrosine under the conditions of this assay

0.5 = Volume (in milliliter) of enzyme used

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

## Enzymatic Assay of PROTEASE

### CALCULATIONS:

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

### UNIT DEFINITION:

One unit will hydrolyze casein to produce peptide equivalent to 1.0  $\mu\text{mole}$  (181  $\mu\text{g}$ ) of Tyrosine per minute at pH 11.0 at 30°C. This protease is an alkaline protease and is approximately twice as active at pH 11.0, as described above, than at the usual assay conditions for protease, pH 7.5 and 37°C. By comparison, P5147 Type XIV protease is only approximately 25% as active at pH 11.0, 30°C.

### FINAL ASSAY CONCENTRATIONS:

In a 3.50 ml reaction mix, the final concentrations are 0.5% (w/v) casein, 9 mM sodium tetraborate, 0.3 mM calcium acetate, and 0.10 - 0.15 unit protease.

### REFERENCE:

Nakanishi, T., Matsumura, Y., Minamiura, N., and Yamamoto, T. (1974) *Agricultural Biological Chemistry* **38**, 37-44

### NOTES:

1. This value was experimentally determined by Sigma.
2. This assay is based on the cited reference.
3. 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.**