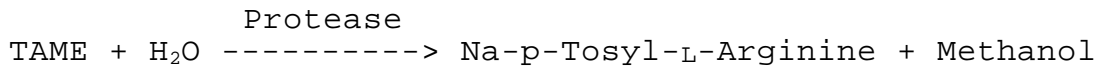


**Enzymatic Assay of PROTEASE
(EC 3.4.21.40)**

PRINCIPLE:



Abbreviation used:

TAME = Na-p-Tosyl-L-Arginine Methyl Ester

CONDITIONS: T = 25°C, pH 8.0, A_{247nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Potassium Phosphate Buffer, pH 8.0 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Dibasic Trihydrate, Sigma Prod. No. P-5504. Adjust to pH 8.0 at 25°C with Reagent B.)
- B. 100 mM Potassium Phosphate Solution
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379.)
- C. 0.80 mM Na-p-Tosyl-L-Arginine Methyl Ester Solution (TAME)
(Prepare 50 ml in Reagent A using Na-p-Tosyl-L-Arginine Methyl Ester, Sigma Prod. No. T-4626.)
- D. Protease Enzyme Solution
(Immediately before use, prepare a solution containing 1.7 - 2.1 units/ml of Protease in cold deionized water.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent C (TAME)	2.90	2.90

Equilibrate to 25°C. Then add:

Reagent D (Enzyme Solution)	0.10	-----
Deionized Water	-----	0.10

Immediately mix by inversion and record the increase in A_{247nm} for approximately 10 minutes. Obtain the $\Delta A_{247nm}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{247nm}/\text{min Test} - \Delta A_{247nm}/\text{min Blank})(3)(df)}{(0.54)(0.1)}$$

3 = Volume (in milliliters) of enzyme used

df = Dilution factor

0.54 = Millimolar extinction coefficient of
Na-p-Tosyl-L-Arginine at 247nm

0.1 = 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 Na-p-tosyl-L-arginine methyl ester per minute at pH 8.0 at 25°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 reaction mix, the final concentrations are 97 mM potassium phosphate, 0.77 mM Na-p-tosyl-L-arginine methyl ester and 0.17 - 0.21 unit protease.

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REFERENCE:

Rick, W. (1974) in *Methods of Enzymatic Analysis*
(Bergmeyer, H.U. ed) 2nd ed., Volume II, pp 1021 - 1024,
Academic Press, Inc., New York, NY

NOTES:

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.