Enzymatic Assay of PROTEASE  
(EC 3.4.21.40)

PRINCIPLE:

Protease
TAME + H₂O --------> Na-p-Tosyl-L-Arginine + Methanol

Abbreviation used:
TAME = Na-p-Tosyl-L-Arginine Methyl Ester

CONDITIONS:  T = 25°C, pH 8.0, A₂₄₇nm, 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.  
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)

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.)

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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
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<tbody>
<tr>
<td>Reagent C (TAME)</td>
<td>2.90</td>
<td>2.90</td>
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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 $A_{247\text{nm}}$ for approximately 10 minutes. Obtain the $\Delta A_{247\text{nm}}$/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

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:

(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 OUR Product or Stock numbers are specified,  
equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of our quality control  
procedure contact our Technical Service Department.