Enzymatic Assay of PROTEASE
(Endoproteinase Lys-C)
from Lysobacter enzymogenes

PRINCIPLE:

\[
\text{N-p-Tosyl-Gly-Pro-Lys-pNA + H}_2\text{O} \xrightarrow{\text{Protease}} \text{N-p-Tosyl-Gly-Pro-Lys + p-Nitroaniline}
\]

Abbreviation used:
pNA = p-Nitroanilide

CONDITIONS:  \( T = 25^\circ\text{C}, \; \text{pH 7.7}, \; A_{405\text{nm}}, \; \text{Light path} = 1 \text{ cm} \)

METHOD:  Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 25 mM Tris HCl Buffer with 1 mM Ethylenediaminetetraacetic Acid, pH 7.7 at 25°C
   (Prepare 100 ml in deionized water using Trizma Base, and Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate. Adjust to pH 7.7 at 25C with 1 M HCl.)

B. 14 mM N-p-Tosyl-Gly-Pro-Lys p-Nitroanilide Substrate Solution
   (Prepare 1 ml in Reagent A using N-p-Tosyl-Gly-Pro-Lys p-Nitroanilide.)

C. Protease Enzyme Solution
   (Immediately before use, prepare a solution containing 0.075 - 0.15 unit/ml of Protease in cold Reagent A.)

PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.70</td>
<td>2.70</td>
</tr>
<tr>
<td>Reagent B (Substrate Solution)</td>
<td>0.15</td>
<td>0.15</td>
</tr>
</tbody>
</table>
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PROCEDURE: (continued)

Mix by inversion and equilibrate to 25°C. Monitor the A405nm until constant, using a suitably thermostatted spectrophotometer. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (Enzyme Solution)</td>
<td>0.10</td>
<td>------</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>------</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the increase in A405nm for approximately 5 minutes. Obtain the ΔA405nm/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(\Delta A_{405\text{nm}}/\text{min Test} - \Delta A_{405\text{nm}}/\text{min Blank})(2.95)(df)}{(10.4)(0.1)}
\]

2.95 = Volume (in milliliters) of assay
df = Dilution factor
10.4 = Millimolar extinction coefficient of p-nitroaniline at 405 nm
0.1 = Volume (in milliliter) of enzyme used

UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of N-p-Tosyl-Gly-Pro-Lys p-Nitroanilide per minute at 7.7 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 2.95 ml reaction mix, the final concentrations are 25 mM Tris, 1 mM ethylenediaminetetraacetic acid, 0.71 mM N-p-tosyl-gly-pro-lys p-nitroanilide, and 0.0075 – 0.015 unit protease.

REFERENCE:

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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.