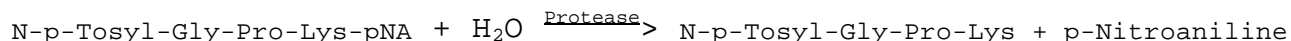


**Enzymatic Assay of PROTEASE
(Endoproteinase Lys-C)
from Lysobacter enzymogenes**

PRINCIPLE:



Abbreviation used:

pNA = p-Nitroanilide

CONDITIONS: T = 25°C, pH 7.7, A_{405nm}, Light path = 1 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, Sigma Prod. No. T-1503 and Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS. Adjust to pH 7.7 at 25°C 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, Sigma Prod. No. T-6140.)
- 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:

| | <u>Test</u> | <u>Blank</u> |
|--------------------------------|-------------|--------------|
| Reagent A (Buffer) | 2.70 | 2.70 |
| Reagent B (Substrate Solution) | 0.15 | 0.15 |

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PROCEDURE: (continued)

Mix by inversion and equilibrate to 25°C. Monitor the $A_{405\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

| | <u>Test</u> | <u>Blank</u> |
|-----------------------------|-------------|--------------|
| Reagent C (Enzyme Solution) | 0.10 | ----- |
| Reagent A (Buffer) | ----- | 0.10 |

Immediately mix by inversion and record the increase in $A_{405\text{nm}}$ for approximately 5 minutes. Obtain the $\Delta A_{405\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

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:

Elliot, B.W., Jr. and Cohen, C. (1986) *Journal of Biological Chemistry* **261**, 11259-11265

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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.