

**Enzymatic Assay of PROLINE IMINOPEPTIDASE
(EC 3.4.11.5)**

PRINCIPLE:

L-Proline p-Nitroanilide $\xrightarrow{\text{PIP}}$ L-Proline + p-Nitroaniline

Abbreviations used:

PIP = Proline Iminopeptidase

CONDITIONS: T = 30°C, pH = 8.0, A_{410nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 100 mM Tris HCl Buffer, pH 8.0 at 30°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.2 at 30°C with 1 M HCl.)
- B. 1.0 mM L-Proline p-Nitroanilide Solution (PPNA)
(Prepare 10 ml in deionized water using L-Proline p-Nitroanilide, Trifluoroacetic Acid, Salt, Sigma Prod. No. P-5267.)
- C. 1000 mM Sodium Acetate Buffer, pH 4.0 at 30°C (Acet)
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 4.0 at 30°C with 5 N HCl.)
- D. Proline Iminopeptidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.075 - 0.150 unit/ml of Proline Iminopeptidase in cold Reagent A.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	1.00	1.25

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PROCEDURE: (continued)

	<u>Test</u>	<u>Blank</u>
Reagent B (PPNA)	0.25	0.25

Equilibrate to 30°C. Then add:

Reagent D (Enzyme Solution)	0.25	-----
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Immediately mix by swirling and incubate for exactly 10 minutes. Then add:

Reagent C (Acet)	0.50	0.50
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Mix by swirling and transfer to suitable cuvettes. Record the $A_{410\text{nm}}$ for both the Test and Blank using a suitable spectrophotometer.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{410\text{nm}} \text{ Test} - A_{410\text{nm}} \text{ Blank})(2.0)(\text{df})}{(10)(5.57)(0.25)}$$

2.0 = Total volume (in milliliters) of assay

df = Dilution factor

10 = Time of assay (in minutes)

5.57 = Millimolar extinction coefficient of p-Nitroaniline under the conditions of this assay

0.25 = Volume (in milliliter) 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 proline p-nitroanilide per minute at pH 8.0 at 30°C.

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FINAL ASSAY CONCENTRATION:

In a 1.50 ml reaction mix, the final concentrations are 83 mM Tris, 0.17 mM L-proline p-nitroanilide and 0.019 - 0.038 unit proline iminopeptidase.

REFERENCE:

Yoshimoto, T. and Tsuru, D. (1985) *Journal of Biochemistry* **97**, 1447 - 1485.

NOTES:

1. This assay is based on the cited reference.
2. All products and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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