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:**  $T = 30^\circ C$, pH = 8.0, $A_{410\text{nm}}$, 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. 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.)

C. 1000 mM Sodium Acetate Buffer, pH 4.0 at 30°C (Acet)  
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate. 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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
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</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>1.00</td>
<td>1.25</td>
</tr>
</tbody>
</table>
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**PROCEDURE:** (continued)

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B (PPNA)</td>
<td>0.25</td>
<td>0.25</td>
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</tbody>
</table>

Equilibrate to 30°C. Then add:

| Reagent D (Enzyme Solution) | 0.25   | ------ |

Immediately mix by swirling and incubate for exactly 10 minutes. Then add:

| Reagent C (Acet) | 0.50   | 0.50  |

Mix by swirling and transfer to suitable cuvettes. Record the $A_{410nm}$ for both the Test and Blank using a suitable spectrophotometer.

**CALCULATIONS:**

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

- 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:**


**NOTES:**

1. This assay is based on the cited reference.

2. All products and stock numbers, unless otherwise indicated, are **OUR** product and stock numbers.

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