Enzymatic Assay of PYROGLUTAMATE AMINOPEPTIDASE  
(EC 3.4.19.3)

PRINCIPLE:

\[ \text{p Glu-ß-Naphthylamide} + \text{H}_2\text{O} \xrightarrow{\text{PGLUA}} \text{p Glu} + \text{ß-Naphthylamine} \]

\[ \text{ß-Naphthylamine} + \text{NaNO}_2 \xrightarrow{\text{H}^+} \text{Diazo Reagent} \]

\[ \text{Diazo Reagent} + \text{N-(l-Naphthyl)-Ethylenediamine} \rightarrow \text{Blue Azo Dye} \]

Abbreviations:
- \( \text{p Glu} = \text{l-Pyroglutamic acid} \)
- \( \text{PGLUA} = \text{Pyroglutamate Aminopeptidase} \)
- \( \text{p Glu-ß-Naphthylamide} = \text{l-Pyroglutamic acid ß-Naphthylamide} \)

CONDITIONS:  \( T = 37°C, \ pH 8.0, \ A_{580nm}, \ \text{Light path = 1 cm} \)

METHOD:  Colorimetric

REAGENTS:

A. 100 mM Potassium Phosphate Buffer with 10 mM Ethylenediaminetetraacetic Acid, 5.0% (v/v) Glycerol, and 5.0 mM Dithiothreitol, pH 8.0 at 37°C  
(Prepare 100 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate, Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Glycerol and DL-Dithiothreitol.  
Adjust to pH 8.0 at 37°C with 1 M HCl.)

B. Methanol  
(Use Methanol, Absolute.)

C. 22 mM l-Pyroglutamic Acid ß-Naphthylamide Solution  
(p Glu-ß-Nap)  
(Prepare 5 ml in Reagent B using l-Pyroglutamic Acid ß-Naphthylamide.)
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REAGENTS: (continued)

D. 25% (w/v) Trichloroacetic Acid Solution (TCA)
   (Prepare 25 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution, approximately 100% (w/v).)

E. 0.1% (w/v) Sodium Nitrite Solution (NaNO₂)
   (Prepare 10 ml in deionized water using Sodium Nitrite.)

F. 0.5% (w/v) Ammonium Sulfamate Solution (NH₄ Sulf)
   (Use Ammonium Sulfamate, 0.5% (w/v).)

G. 95% (v/v) Ethanol
   (Prepare 125 ml in deionized water using Ethyl Alcohol, Denatured.)

H. 0.05% (w/v) N-1-Naphthylethylenediamine Solution (NED)
   (Prepare by adding 110 ml of Reagent G to the contents of 1 bottle of N-1-Naphthylethylenediamine.)

I. 0.0018% (w/v) β-Naphthylamine Standard Solution (Std)
   (Use LAP Calibration Solution.)

J. Pyroglutamate Aminopeptidase Enzyme Solution
   (Immediately before use, prepare a solution containing 35 - 70 units/ml of Pyroglutamate Aminopeptidase in cold Reagent A.)

PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Reagent C (p Glu-β-Nap)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Mix by swirling and equilibrate to 37°C. Then add:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent J (Enzyme Solution)</td>
<td>0.10</td>
</tr>
</tbody>
</table>
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(EC 3.4.19.3)

PROCEDURE:  (continued)

Immediately mix by swirling and incubate at 37°C for exactly 15 minutes. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent D (TCA)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Reagent J (Enzyme Solution)</td>
<td>------</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Mix by swirling.

COLORIMETRIC ASSAY:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Std 4</th>
<th>Std 5</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Solution</td>
<td>1.00</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
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</tr>
<tr>
<td>Blank Solution</td>
<td>----</td>
<td>1.00</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Reagent I (Std)</td>
<td>----</td>
<td>----</td>
<td>0.10</td>
<td>0.20</td>
<td>0.30</td>
<td>0.50</td>
<td>0.80</td>
<td>----</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>----</td>
<td>----</td>
<td>0.90</td>
<td>0.80</td>
<td>0.70</td>
<td>0.50</td>
<td>0.20</td>
<td>1.00</td>
</tr>
<tr>
<td>Reagent E (NaNO₃)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

Mix quickly by swirling and incubate at room temperature for 3 minutes. Then add:

<table>
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<tr>
<th></th>
<th>Test</th>
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<th>Std 1</th>
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<th>Std 3</th>
<th>Std 4</th>
<th>Std 5</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent F (NH₄ Sulf)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
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<th>Std 3</th>
<th>Std 4</th>
<th>Std 5</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent H (NED)</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
</tbody>
</table>

Mix quickly by swirling and incubate at room temperature for 45 minutes. Transfer the solutions to suitable cuvettes and record the A₅₈₀nm for the Test, Test Blank, Standards, and Standard Blank using a suitable spectrophotometer.

CALCULATIONS:
Standard Curve:

$$\Delta A_{580\text{nm}} \text{ Standard} = A_{580\text{nm}} \text{ Standard} - A_{580\text{nm}} \text{ Standard Blank}$$

Prepare a standard curve by plotting the $$\Delta A_{580\text{nm}} \text{ Standard}$$ vs nanomoles of β-Naphthylamine.
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CALCULATIONS:  (continued)

Sample Determination:

\[ \Delta A_{580nm} \text{ Sample} = A_{580nm} \text{ Test} - A_{580nm} \text{ Blank} \]

Determine the nanomoles of \( \beta \)-Naphthylamine liberated using the standard curve.

\[
\text{Units/ml enzyme} = \frac{\text{(nanomoles of } \beta \text{-Naphthylamine liberated)}(2.2)(df)}{(15)(0.1)}
\]

2.2 = Total volume (in milliliters) of stopped reaction  
\( df \) = Dilution factor
15 = Time (in minutes) of assay as per the Unit Definition
0.1 = 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 nanomole of \( L \)-pyroglutamic acid \( \beta \)-naphthylamide to \( L \)-pyroglutamic acid and \( \beta \)-naphthylamine per minute at pH 8.0 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 1.20 ml reaction mix, the final concentrations are 92 mM potassium phosphate, 9.2 mM ethylenediaminetetraacetic acid, 4.6% (v/v) glycerol, 4.6 mM \( DL \)-dithiothreitol, 1.8 mM \( L \)-pyroglutamic acid \( \beta \)-naphthylamide, and 3.5 - 7.0 unit pyroglutamate aminopeptidase.

REFERENCE:


NOTES:

1. This assay is based on the cited reference.
Enzymatic Assay of PYROGLUTAMATE AMINOPEPTIDASE  
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PROCEDURE: (continued)

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.