Enzymatic Assay of CARBOXYPEPTIDASE P  
(EC 3.4.17.16)

PRINCIPLE:

\[ \text{N-CBZ-Glu-Tyrosine} + \text{H}_2\text{O} \xrightarrow{\text{Carboxypeptidase P}} \text{N-CBZ-L-Glutamic Acid} + \text{L-Tyrosine} \]

Abbreviation used:
N-CBZ = N-Carbobenzoxy

CONDITIONS:  \( T = 30^\circ\text{C}, \text{pH} = 3.7, A_{570\text{nm}}, \text{Light path} = 1 \text{ cm} \)

METHOD:  Colorimetric

REAGENTS:

A. 50 mM Sodium Acetate Buffer with 0.02% (v/v) Triton X-100, pH 3.7 at 30°C  
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate and Triton X-100.  Adjust the pH to 3.7 at 30°C with 1 M HCl.)

B. 300 mM Sodium Hydroxide Solution (NaOH)  
(Prepare 100 ml in deionized water using Sodium Hydroxide Solution, 1.0 Normal.)

C. 2.5% (v/v) Acetic Acid Solution (HOAC)  
(Prepare 100 ml in deionized water using Acetic Acid, Glacial.)

D. 500 mM Sodium Citrate Solution, pH 5.0 at 30°C (Sod Cit)  
(Prepare 100 ml in deionized water using Citric Acid, Free Acid, Anhydrous.  Adjust the pH to 5.0 at 30°C using 1 M NaOH.)

E. Ninhydrin Color Reagent (NCR)  
(Prepare 60 ml by adding 0.5 g Ninhydrin, to 59 ml of Ethylene Glycol Monomethyl Ether.  Then add 1 ml of Reagent F.)
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REAGENTS: (continued)

F. 10 mM Potassium Cyanide Solution (KCN)  
(Prepare 2 ml in deionized water using Potassium Cyanide.)

G. 65% (v/v) Ethanol (EtOH)  
(Prepare 25 ml in deionized water using Ethyl Alcohol HPLC Grade.)

H. 0.1 mM Tyrosine Standard Solution (Std Soln)  
(Prepare 10 ml in deionized water using L-Tyrosine, Free Base. Heat gently to dissolve.)

I. 1 mM N-CBZ-Glu-Tyrosine Substrate Solution  
(Prepare 10 ml in Reagent A using N-CBZ-Glu-Tyr. Heat gently to dissolve.)

K. Carboxypeptidase P Enzyme Solution  
(Immediately before use, prepare a solution containing 0.03 – 0.06 unit/ml Carboxypeptidase P in Reagent A.)

PROCEDURE:

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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent I (Substrate Soln)</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.30</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Mix by swirling and equilibrate to 30°C. Then add:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent K (Enzyme Soln)</td>
<td>0.20</td>
<td>------</td>
</tr>
</tbody>
</table>

Mix by swirling and incubate at 30°C for exactly 20 minutes. Then add:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B (NaOH)</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Reagent K (Enzyme Soln)</td>
<td>------</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Mix by swirling and incubate at 30°C for 30 minutes.
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COLOR DEVELOPMENT:

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

<table>
<thead>
<tr>
<th>Std</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>Std Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Solution</td>
<td>1.20</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Blank Solution</td>
<td>---</td>
<td>1.20</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Reagent H (Std Soln)</td>
<td>---</td>
<td>---</td>
<td>0.20</td>
<td>0.40</td>
<td>0.80</td>
<td>1.00</td>
<td>1.20</td>
<td>---</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>---</td>
<td>---</td>
<td>1.00</td>
<td>0.80</td>
<td>0.40</td>
<td>0.20</td>
<td>---</td>
<td>1.20</td>
</tr>
<tr>
<td>Reagent C (HOAC)</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Reagent D (Sod Cit)</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>
<tr>
<td>Reagent E (NCR)</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>1.20</td>
</tr>
</tbody>
</table>

Mix by swirling and place vented caps on each container. Place the containers in a boiling water bath for 15 minutes. Cool on ice, and then add:

<table>
<thead>
<tr>
<th>Std</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>Std Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent G (EtOH)</td>
<td>0.60</td>
<td>0.60</td>
<td>0.60</td>
<td>0.60</td>
<td>0.60</td>
<td>0.60</td>
<td>0.60</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Mix by swirling and transfer the contents of the containers to suitable cuvettes. Read the absorbance at 570nm for each of the cuvettes using a suitable spectrophotometer.

CALCULATION:

Standard Curve:

\[ \Delta A_{570nm} \text{ Standard} = A_{570nm} \text{ Standard} - A_{570nm} \text{ Standard Blank} \]

Plot the \( \Delta A_{570nm} \) Standard vs µmoles Tyrosine.

Sample Determination:

\[ \Delta A_{570nm} \text{ Sample} = A_{570nm} \text{ Test} - A_{570nm} \text{ Test Blank} \]

Determine the µmoles of Tyrosine liberated using the standard curve.

\[
\text{Units/ml enzyme} = \frac{(\mu\text{mole Tyrosine liberated}) \ (df)}{(20) (0.2)}
\]

df = Dilution factor  
20 = Time of assay (in minutes) as per the Unit Definition
0.2 = Volume (in milliliters) of enzyme used
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CALCULATION: (continued)

\[
\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}
\]

\[
\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}
\]

UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of N-CBZ-Glu-Tyr to N-CBZ-L-glutamic acid and L-tyrosine per minute at pH 3.7 at 30°C.

FINAL CONCENTRATION:

In a 1.00 ml reaction mix, the final concentrations are 50 mM sodium acetate, 0.02% (v/v) Triton® X-100, 0.5 mM N-CBZ-Glu-Tyrosine, and 0.006 - 0.012 unit carboxypeptidase P.

REFERENCES:


NOTES:

1. Triton is a registered trademark of Union Carbide Chemicals and Plastics Co., Inc.

2. Where OUR Product or Stock numbers are specified, equivalent reagents may be substituted.

3. This assay is based on the cited reference.

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