Enzymatic Assay of CARBOXYPEPTIDASE INHIBITOR

PRINCIPLE:

Carboxypeptidase Inhibitor will inhibit the reaction shown below:

\[
\text{Carboxypeptidase A} \quad \text{Hippuryl-}L\text{-Phe} + H_2O \quad \rightarrow \quad \text{Hippuric acid} + L\text{-Phenylalanine}
\]

CONDITIONS: \( T = 25^\circ C, \ \text{pH} = 7.5, A_{254nm}, \ \text{Light path} = 1 \text{ cm} \)

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 20 mM Tris HCl Buffer with 500 mM Sodium Chloride, pH 7.5 at 25°C
   (Prepare 100 ml in deionized water using Trizma Base and Sodium Chloride. Adjust to pH 7.5 at 25°C with 1 M HCl.)

B. 1.0 mM Hippuryl-L-Phenylalanine Solution (Hippuryl-L-Phe)
   (Prepare 30 ml in Reagent A using Hippuryl-Phe. Dissolution may require 30 minutes.)

C. Carboxypeptidase A Enzyme Solution (CPA)
   (Immediately before use, prepare a solution containing approximately 0.1 mg/ml \( \forall 50\% \) of Carboxypeptidase A, in cold Reagent A. The protein concentration should be determined using the \( E_{280nm}^{0.1} = 1.88 \).)

D. Carboxypeptidase Inhibitor (CPI)
   (Immediately before use, prepare a solution in Reagent A containing Carboxypeptidase Inhibitor so that a 0.05 ml aliquot produces 20 - 70% inhibition of Carboxypeptidase A.)

PROCEDURE:

Preincubate 0.05 ml of Reagent D (CPI) and 0.05 ml of Reagent C (CPA) at 25°C for 30 seconds.
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PROCEDURE: (continued)

Pipette in (milliliters) the following reagents into suitable quartz cuvettes.

<table>
<thead>
<tr>
<th></th>
<th>CPI/CPA</th>
<th>CPA</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B (Hippuryl-L-Phe)</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>CPI/CPA Mixture</td>
<td>0.10</td>
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</tr>
<tr>
<td>Reagent C (CPA)</td>
<td>----</td>
<td>0.05</td>
<td>------</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>----</td>
<td>0.05</td>
<td>0.10</td>
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</table>

Immediately mix by inversion and record the increase in $A_{254\text{nm}}$ for approximately 3 minutes using air as the reference. Obtain the $\Delta A_{254\text{nm}}$/minute using the maximum linear rate for the Test, Control, and Blank.

CALCULATIONS:

A. 

\[ \text{% Inhibition} = 1 - \left( \frac{\Delta A_{254\text{nm}}/\text{min CPI/CPA}}{\Delta A_{254\text{nm}}/\text{min CPA}} \right) \times 100 \]

B. 

\[
\text{Micrograms of CPI/Reaction Mixture} = \frac{50 \, \mu l \times \mu g/\mu l \text{ CPI (Lowry Protein)}^2}{\text{dilution (factor)}}
\]

C. 

Since the assay is linear at 20 - 70% inhibition, use the following equation to determine how many micrograms of CPI are needed to produce 50% inhibition.

EX:

\[
\mu g \text{ CPI} \quad \times \quad \%	ext{Inhibition} \quad = \quad 50
\]

D. 

\[
\text{Micrograms of CPA/Reaction Mixture} = 50 \, \mu l \times \mu g/\mu l \text{ CPA (Use } E_{280\text{nm}}^{0.1\%} = 1.88)
\]
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CALCULATIONS: (continued)

E.

\[
\text{Units/\(\mu\)g CPI protein} = \frac{\mu\text{g CPA/RM}}{\mu\text{g CPI needed to produce 50\% inhibition}}
\]

RM = Reaction Mixture

SPECIFICATION:

One \(\mu\)g of inhibitor protein will inhibit the activity of 2 - 8 \(\mu\)g of carboxypeptidase A (C9268) by 50\% using hippuryl-L-phenylalanine as substrate at pH 7.5 at 25\°C.

FINAL ASSAY CONCENTRATION:

In a 3.10 ml reaction mix, the final concentrations are 20 mM Tris, 500 mM sodium chloride, 0.97 mM hippuryl-L-phenylalanine, approximately 0.005 mg carboxypeptidase A, and varying amounts of carboxypeptidase inhibitor.

REFERENCE:


NOTES:

1. Determine the protein concentration of the Carboxypeptidase Inhibitor by the Lowry Method. Dilutions must be made so that the sample does not exceed 0.025 mg of bovine serum albumin equivalents.

2. 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.