Enzymatic Assay of CARBOXYPEPTIDASE Y

PRINCIPLE:

\[ \text{N-CBZ-Phe-Ala} + \text{H}_2\text{O} \xrightarrow{\text{Carboxypeptidase Y}} \text{N-CBZ-}{L}\text{-Phe} + {L}\text{-Alanine} \]

Abbreviations used:
N-CBZ-Phe-Ala = N-Carbobenzyo-\(L\)-Phenylalanine-\(L\)-Alanine
N-CBZ-\(L\)-Phe = N-Carbobenzyo-\(L\)-Phenylalanine

CONDITIONS: \(T = 25^\circ\text{C}, \text{pH} = 6.75, A_{230\text{nm}}, \text{Light path} = 1 \text{ cm}\)

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 50 mM 2-[N-Morpholino]Ethanesulfonic Acid (MES) Buffer, pH 6.75 at 25°C
   (Prepare 200 ml in deionized water using MES, Free Acid,
   Sigma Prod. No. M-8250. Adjust to pH 6.75 at 25°C with
   1 M NaOH.)

B. 2.0 mM N-CBZ-\(L\)-Phenylalanine-\(L\)-Alanine Solution
   (CBZ-Phe-Ala)
   (Prepare 100 ml in Reagent A using N-CBZ-Phe-Ala, Sigma Prod. No. C-1634. Facilitate solubilization by
   first dissolving in 2 ml of Methanol, Sigma Prod. No. M-3641. Adjust to pH 6.75 at 25°C with 1 M
   HCl or 1 M NaOH, if necessary.)

C. Carboxypeptidase Y Enzyme Solution
   (Immediately before use prepare a solution containing
   36 - 72 units/ml of Carboxypeptidase Y in cold
   Reagent A.)
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PROCEDURE:

Pipet (in milliliters) the following reagents into suitable quartz 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>------</td>
<td>0.01</td>
</tr>
<tr>
<td>Reagent B (CBZ-Phe-Ala)</td>
<td>3.00</td>
<td>3.00</td>
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</table>

Mix by inversion and equilibrate to 25°C. Monitor the \(A_{230\text{nm}}\) until constant, using a suitably thermostatted spectrophotometer. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (Enzyme Solution)</td>
<td>0.01</td>
<td>------</td>
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Immediately mix by inversion and record the decrease in \(A_{230\text{nm}}\) for approximately 5 minutes. Obtain the \(\Delta A_{230\text{nm}}/\text{minute}\) using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

\[
\text{Units/mg enzyme} = \frac{\Delta A_{230\text{nm}}/\text{min Test} - \Delta A_{230\text{nm}}/\text{min Blank}}{(0.1915) (\text{mg enzyme/ml RM})}
\]

0.1915 = Millimolar extinction coefficient of N-CBZ-Phe-Ala at 230 nm

RM = Reaction Mix

UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of N-CBZ-Phe-Ala to N-CBZ-\(\text{L}\)-phenylalanine and \(\text{L}\)-alanine per minute at pH 6.75 at 25°C, based on \(E_{230\text{M}}^M = 191.5\).

FINAL ASSAY CONCENTRATION:

In a 3.01 ml reaction mix, the final concentrations are 50 mM MES, 2.0 mM N-CBZ-Phe-Ala, 2% (w/v) methanol, and 0.36 - 0.72 unit carboxypeptidase Y.

NOTES:
1. All product 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.