Enzymatic Assay of CARBOXYPEPTIDASE Y

PRINCIPLE:

N-CBZ-Phe-Ala + H_2O $\xrightarrow{\text{Carboxypeptidase Y}}$ N-CBZ-$_L$-Phe + $_L$-Alanine

Abbreviations used:
N-CBZ-Phe-Ala = N-Carbobenzoxy-$_L$-Phenylalanine-$_L$-Alanine
N-CBZ-$_L$-Phe = N-Carbobenzoxy-$_L$-Phenylalanine

CONDITIONS: T = 25°C, pH = 6.75, A_{230nm}, Light path = 1 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:

	Test	Blank
Reagent A (Buffer)	------	0.01
Reagent B (CBZ-Phe-Ala)	3.00	3.00

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

	Test	Blank
Reagent C (Enzyme Solution)		0.01

Immediately mix by inversion and record the decrease in A_{230nm} for approximately 5 minutes. Obtain the ΔA_{230nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

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-L-phenylalanine and L-alanine per minute at pH 6.75 at 25°C, based on $E^{M}_{230}1 = 191.5$.

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.