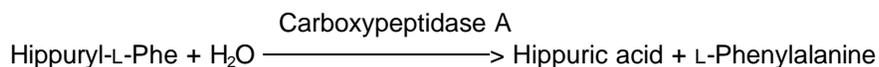# Enzymatic Assay of CARBOXYPEPTIDASE INHIBITOR

PRINCIPLE:

Carboxypeptidase Inhibitor will inhibit the reaction shown below:

$$\text{Hippuryl-L-Phe} + H_2O \xrightarrow{\quad \text{Carboxypeptidase A} \quad} \text{Hippuric acid} + \text{L-Phenylalanine}$$

CONDITIONS: T = 25°C, pH = 7.5, A_{254nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A.   20 mM Tris HCl Buffer with 500 mM Sodium Chloride, pH 7.5 at 25EC
     (Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503, and Sodium Chloride, Sigma Prod. No. S-9625. 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, Sigma Prod. No. H-6875. Dissolution may require 30 minutes.)

C.   Carboxypeptidase A Enzyme Solution (CPA)
     (Immediately before use, prepare a solution containing approximately 0.1 mg/ml ∀ 50% of Carboxypeptidase A, Sigma Prod. No. C-0386, 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.

	CPI/CPA Test	CPA Control	Blank
Reagent B (Hippuryl-L-Phe)	3.00	3.00	3.00
CPI/CPA Mixture	0.10	------	------
Reagent C (CPA)	------	0.05	------
Reagent A (Buffer)	------	0.05	0.10

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

CALCULATIONS:

A.

% Inhibition = 1 - (ΔA_{254nm}/min CPI/CPA ÷ ΔA_{254nm}/min CPA) X 100

B.

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

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:

$$\frac{\mu g \ CPI}{\% \ \text{Inhibition}} = \frac{X}{50}$$

D.

Micrograms of CPA/Reaction Mixture = 50 μl x $\mu g/\mu l$ CPA (Use $E_{280nm}^{0.1\%}$= 1.88)

CALCULATIONS: (continued)

E.

$$\text{Units/µg CPI protein} = \frac{\text{µg CPA/RM}}{\text{µg CPI needed to produce 50\% inhibition}}$$

RM = Reaction Mixture

SPECIFICATION:

One µg of inhibitor protein will inhibit the activity of 2 - 8 µg 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:

Hass, G.M. and Ryan, C.A. (1981) *Methods in Enzymology* **80**, 778-791

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 Sigma's quality control procedure contact our Technical Service Department.