Enzymatic Assay of CARBOXYPEPTIDASE P
(EC 3.4.17.16)

PRINCIPLE:

N-CBZ-Glu-Tyrosine + H_2O $\xrightarrow{\text{Carboxypeptidase P}}$ N-CBZ-L-Glutamic Acid + L-Tyrosine

Abbreviation used:
N-CBZ = N-Carbobenzoxy

CONDITIONS: T = 30°C, pH = 3.7, A_{570nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

A. 50 mM Sodium Acetate Buffer with 0.02% (v/v) Triton[1] X-100, pH 3.7 at 30°C
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625 and Triton[1] X-100, Sigma Stock No. 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, Sigma Stock No. 930-65.)

C. 2.5% (v/v) Acetic Acid Solution (HOAC)
(Prepare 100 ml in deionized water using Acetic Acid, Glacial, Sigma Prod. No. A-6283.)

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, Sigma Prod. No. C-0759. 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, Sigma Prod. No. N-4876, to 59 ml of Ethylene Glycol Monomethyl Ether, Sigma Prod. No. E-5378. 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, Sigma Stock No. 20,781-0.)

G. 65% (v/v) Ethanol (EtOH)
(Prepare 25 ml in deionized water using Ethyl Alcohol HPLC Grade, Sigma Stock No. 27074-1.)

H. 0.1 mM Tyrosine Standard Solution (Std Soln)
(Prepare 10 ml in deionized water using L-Tyrosine, Free Base, Sigma Prod. No. T-3754. Heat gently to dissolve.)

I. 1 mM N-CBZ-Glu-Tyrosine Substrate Solution
(Prepare 10 ml in Reagent A using N-CBZ-Glu-Tyr, Sigma Prod. No. C-0257. 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:

	Test	Blank
Reagent I (Substrate Soln)	0.50	0.50
Reagent A (Buffer)	0.30	0.30

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

Reagent K (Enzyme Soln)		0.20

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

Reagent B (NaOH)	0.20	0.20
Reagent K (Enzyme Soln)		0.20

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:

	Test	Blank	Std 1	Std 2	Std 3	Std 4	Std 5	Std Blank
Test Solution	1.20	---	---	---	---	---	---	---
Blank Solution	---	1.20	---	---	---	---	---	---
Reagent H (Std Soln)	---	---	0.20	0.40	0.80	1.00	1.20	---
Deionized Water	---	---	1.00	0.80	0.40	0.20	---	1.20
Reagent C (HOAC)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Reagent D (Sod Cit)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Reagent E (NCR)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.20

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:

Reagent G (EtOH)	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60

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:

ΔA_{570nm} Standard = A_{570nm} Standard - A_{570nm} Standard Blank

Plot the ΔA_{570nm} Standard vs µmoles Tyrosine.

Sample Determination:

ΔA_{570nm} Sample = A_{570nm} Test - A_{570nm} 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[1] X-100, 0.5 mM N-CBZ-Glu-Tyrosine, and 0.006 - 0.012 unit carboxypeptidase P.

REFERENCES:

Umetsu, H., Abe, M., Sugawara, Y. and Nakai, T. (1981) *Food Chemistry* **7**, 125-138.

NOTES:

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

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

3. This assay is based on the cited reference.