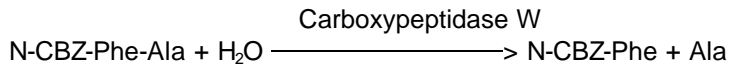


**Enzymatic Assay of CARBOXYPEPTIDASE W
(E.C. 3.4.16.1)**

PRINCIPLE:



Abbreviation used:

N-CBZ = N-Carbobenzoxy

CONDITIONS: T = 30°C, pH = 4.0, $A_{570\text{nm}}$, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 50 mM Sodium Acetate Buffer, pH 4.0 at 30°C (Enz Dil)
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 4.0 at 30°C with 5 M HCl.)
- B. 500 mM Citrate Buffer, pH 5.0 at 30°C (Cit Buffer)
(Prepare 100 ml in deionized water using Citric Acid, Free Acid, Anhydrous, Sigma Prod. No. C-0759. Adjust to pH 5.0 at 30°C with 5 M NaOH.)
- C. 500 mM Sodium Acetate Buffer, pH 6.5 at 30°C.
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 6.5 at 30°C with 1 M HCl.)
- D. 2 mM N-CBZ-Phe-Ala Solution (Substrate)
(Prepare 10 ml in Reagent A using N-CBZ-Phe-Ala, Sigma Prod. No. C-1634. Dissolve by heating in a boiling water bath and mix by swirling.)
- E. 1 mM L-Tyrosine Standard Solution (Std Soln)
(Prepare 10 ml in deionized water using L-Tyrosine, Free Base, Sigma Prod. No. T-3754. Heat gently (do not boil) until the tyrosine dissolves and cool to room temperature.)

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REAGENTS: (continued)

- F. Ninhydrin Color Reagent (NCR)
(Prepare 100 ml by dissolving 2 g of Ninhydrin, Sigma Prod. No. N-4876 and 300 mg of Hydrindantin, Sigma Prod. No. H-2003 in 75 ml of Ethylene Glycol Monomethyl Ether, Sigma Prod. No. E-5378. Note: Bubble N₂ gas through the Ethylene Glycol Monomethyl Ether before adding the reagents. Mix by swirling and bubble N₂ gas through the solution (the solution should be bright yellow). Add 25 ml of Reagent C and mix by swirling. The solution should turn red. Place into another amber bottle and purge with N₂ gas. Seal the bottle tightly. The ninhydrin color reagent must be in the reduced form (red in color) for use in this assay. The solution is stable for 2 - 3 days.)
- G. Carboxypeptidase W Enzyme Solution
(Immediately before use, prepare a solution containing 0.075 - 0.15 unit/ml of Carboxypeptidase W in cold Reagent A.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable containers:

	<u>Test</u>	<u>Blank</u>
Reagent D (Substrate)	0.50	0.50
Reagent A (Enz Dil)	0.40	0.40

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

Reagent G (Enz Soln)	0.10	-----
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Mix by swirling and incubate for exactly 20 minutes at 30°C.

COLOR DEVELOPMENT:

Pipette (in milliliters) the following reagents into suitable containers:

	<u>Test</u>	<u>Blank</u>	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Std 4</u>	<u>Std 5</u>	<u>Std Blank</u>
Reagent F (NCR)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Reagent B (Cit Buffer) 3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	
Test Solution	1.00	---	---	---	---	---	---	---
Blank Solution	---	0.90	---	---	---	---	---	---
Reagent G (Enz Soln)	---	0.10	---	---	---	---	---	---
Reagent E (Std Soln)	---	---	0.05	0.10	0.15	0.25	0.30	---
Deionized Water	---	---	0.95	0.90	0.85	0.75	0.70	1.00

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COLOR DEVELOPMENT: (continued)

Mix by swirling and place vented caps on each container. Place these containers in a boiling water bath for 15 minutes. Cool on ice for 10 minutes. Transfer the contents of the containers to suitable cuvettes. Record the absorbance at 570 nm for each of the cuvettes using a suitable spectrophotometer.

CALCULATION:

Standard Curve:

$$\Delta A_{570\text{nm}} \text{ Standard} = A_{570\text{nm}} \text{ Standard} - A_{570\text{nm}} \text{ Standard Blank}$$

Plot the $\Delta A_{570\text{nm}}$ Standard vs $\mu\text{moles Tyrosine}$

Sample Determination:

$$\Delta A_{570\text{nm}} \text{ Sample} = A_{570\text{nm}} \text{ Test} - A_{570\text{nm}} \text{ Sample Blank}$$

Determine the $\mu\text{moles of Tyrosine equivalents}$ using the standard curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{mole of Tyrosine equivalents})(df)}{(20)(0.1)}$$

df = Dilution factor

20 = Time (in minutes) of assay as per the Unit Definition

0.1 = Volume (in milliliter) of enzyme used

$$\text{Unit/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Unit/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will hydrolyze 1.0 μmole of N-CBZ-Phe-Ala to N-CBZ-L-phenylalanine and L-alanine (equivalent in ninhydrin color to 1.0 μmole of tyrosine) per minute at pH 4.0 at 30°C.

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FINAL CONCENTRATION:

In a 1.00 ml reaction mix, the final concentrations are 50 mM sodium acetate, 1 mM N-CBZ-phe-ala and 0.0075 - 0.015 unit carboxypeptidase W.

REFERENCE:

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

NOTES:

1. This assay is based on the cited reference.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.