

Product Information

SIGMA QUALITY CONTROL TEST PROCEDURE Enzymatic Assay of CARBOXYPEPTIDASE A (EC 3.4.17.1)

PRINCIPLE:

Hippuryl-L-Phe + H₂O $\xrightarrow{\text{Carboxypeptidase A}}$ Hippuric acid + L-Phenylalanine

Abbreviations used:

Hippuryl-L-Phe = Hippuryl-L-Phenylalanine

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

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 25 mM Tris HCl Buffer with 500 mM Sodium Chloride, pH 7.5 at 25°C
(Prepare 100 ml in deionized water using Trizma Hydrochloride, Sigma Prod. No. T-3253, and Sodium Chloride, Sigma Prod. No. S-9625. Adjust to pH 7.5 at 25°C with 1 M NaOH.)
- B. 1.0 mM Hippuryl-L-Phenylalanine Solution (Hippuryl-L-Phe)
(Prepare 100 ml in Reagent A using Hippuryl-Phe, Sigma Prod. No. H-6875. Approximately 30 minutes of stirring is required for this product to dissolve completely. **PREPARE FRESH.**)¹
- C. 1.0 M Sodium Chloride Solution (NaCl)
(Prepare 10 ml in deionized water using Sodium Chloride, Sigma Prod. No. S-9625.)
- D. Carboxypeptidase A Enzyme Solution
(Immediately before use, prepare a solution containing 6 - 12 units/ml of Carboxypeptidase A in cold Reagent C.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable quartz cuvettes:

	<u>Test</u>	<u>Blank</u>
Reagent B (Hippuryl-L-Phe)	2.90	2.90

Equilibrate to 25°C. Monitor the A_{254nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent C (NaCl)	-----	0.10
Reagent D (Enzyme Solution)	0.10	-----

Immediately mix by inversion and record the increase in $A_{254\text{nm}}$ for approximately 5 minutes. Obtain the $\Delta A_{254\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{254\text{nm}}/\text{min Test} - \Delta A_{254\text{nm}}/\text{min Blank})(3)(\text{df})}{(0.36)(0.1)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

0.36 = Millimolar extinction coefficient of hippuric acid at 254 nm

0.1 = Volume (in milliliters) of enzyme used

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

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

UNIT DEFINITION:

One unit will hydrolyze 1.0 μmole of hippuryl-L-phenylalanine per minute at pH 7.5 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 24 mM Tris, 0.97 mM hippuryl-L-phenylalanine, 520 mM sodium chloride, and 0.6 - 1.2 units carboxypeptidase A.

REFERENCE:

Bergmeyer, H.U., Gawehn, K., and Grassl, M. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U., ed) Volume I, 2nd ed., 436-437, Academic Press, Inc. New York, NY

NOTES:

1. Hippuryl-L-Phe solution is unstable and must be used within 3 hours.
2. This assay is based on the cited reference.
3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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