Creative Enzymes

Enzymatic Assay of CARBOXYPEPTIDASE B
(EC 3.4.17.2)

PRINCIPLE:

Hippuryl-L-Arg + H_2O $\xrightarrow{\text{Carboxypeptidase B}}$ Hippuric acid + L-Arginine

Abbreviations used:
Hippuryl-L-Arg = Hippuryl-L-Arginine

CONDITIONS: T = 25°C, pH = 7.65, A_{254nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 25 mM Tris HCl Buffer with 100 mM Sodium Chloride, pH 7.65 at 25°C
(Prepare 100 ml in deionized water using Trizma Hydrochloride, and Sodium Chloride. Adjust to pH 7.65 at 25°C with 1 M NaOH.)

B. 1.0 mM Hippuryl-L-Arginine Solution (Hippuryl-L-Arg)
(Prepare 50 ml in Reagent A using Hippuryl-L-Arginine. **PREPARE FRESH.**)

C. Carboxypeptidase B Enzyme Solution
(Immediately before use, prepare a solution containing 4 - 8 units/ml of Carboxypeptidase B in cold deionized water.)

PROCEDURE:

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

	Test	Blank
Reagent B (Hippuryl-L-Arg)	2.90	2.90

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PROCEDURE: (continued)

Equilibrate to 25°C. Monitor the A_{254nm} until constant, using a suitably thermostatted spectrophotometer and Reagent B as the reference.[1] Then add:

	Test	Blank
Deionized Water	------	0.10
Reagent C (Enzyme Solution)	0.10	------

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

CALCULATIONS:

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

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-arginine per minute at pH 7.65 at 25°C.

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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 24 mM Tris, 0.97 mM hippuryl-L-arginine, 97 mM sodium chloride, and 0.4 - 0.8 unit carboxypeptidase B.

REFERENCES:

Folk, J.E., Piez, K.A., Carroll, W.R. and Gladner, J.A. (1960) *J. Biol. Chem*. **235**, 2272-2277

NOTES:

1. The substrate solution has a high initial A_{254nm} which requires the use of Reagent B rather than air as the reference.

2. This assay is based on the cited reference.

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