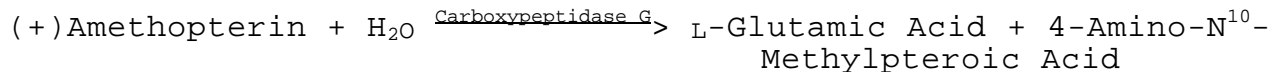


**Enzymatic Assay of CARBOXYPEPTIDASE G  
(EC 3.4.19.9)**

**PRINCIPLE:**



**CONDITIONS:** T = 30°C, pH 7.3, A<sub>320nm</sub>, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

- A. 50 mM Tris HCl Buffer, with 0.1 mM Zinc Chloride, pH 7.3 at 30°C  
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503 and Zinc Chloride, Sigma Prod. No. Z-4875. Adjust to pH 7.3 at 30°C with 1 M HCl.)
- B. 1.8 mM (+)Amethopterin Substrate Solution  
(Prepare 10 ml by dissolving (+)Amethopterin, Hydrate, Sigma Prod. No. A-6770 in 0.1 ml of 1 M NaOH. Dilute to 10 ml with Reagent A.)
- C. Carboxypeptidase G Enzyme Solution  
(Immediately before use, prepare a solution containing 0.3 - 0.6 unit/ml of Carboxypeptidase G in cold deionized water.)

**PROCEDURE:**

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.80	2.80
Reagent B (Substrate Soln)	0.10	0.10

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**PROCEDURE:** (continued)

Mix by inversion and equilibrate to 30°C. Monitor the  $A_{320\text{nm}}$  until constant, using a suitably thermostatted spectrophotometer. Then add:

	<u>Test</u>	<u>Blank</u>
Reagent C (Enzyme Solution)	0.10	-----
Deionized Water	-----	0.10

Immediately mix by inversion and record the decrease in  $\Delta A_{320\text{nm}}$ /minute using the maximum linear rate for both the Test and the Blank.

**CALCULATION:**

$$\text{Units/ml enzyme} = \frac{(\Delta A_{320\text{nm}}/\text{min Test} - \Delta A_{320\text{nm}}/\text{min Blank})(3)(\text{df})}{(8.3)(0.1)}$$

3 = Volume (in milliliters) of assay

df = Dilution factor

8.3 = The difference in the millimolar extinction coefficients between the substrate and product at 320 nm.<sup>1</sup>

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  $\mu\text{mole}$  of L-glutamic acid from (+)amethopterin per minute at pH 7.3 at 30°C.

**FINAL ASSAY CONCENTRATIONS:**

In a 3.00 reaction mix, the final concentrations are 48 mM Tris, 0.1 mM zinc chloride, 0.06 mM (+)amethopterin, and 0.03 - 0.06 unit carboxypeptidase G.

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**REFERENCES:**

- McCullough, J.C., Chabner, B.A. and Bertino, J.R. (1971) *Journal of Biological Chemistry* **246**, 7207-7213.
- Levy, C.C. and Goldman, P. (1967) *Journal of Biological Chemistry* **242**, 2933-2938.

**NOTES:**

1. The difference in the extinction coefficients for (+)amethopterin and 4-amino-N<sup>10</sup>-methylpteroic acid is described in the reference by McCullough, J.L., et al. (1971).
2. This assay is based on the cited references.
3. 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.**