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SIGMA QUALITY CONTROL TEST PROCEDURE
Enzymatic Assay of CARBOXYPEPTIDASE G
(EC 3.4.7.11)

PRINCIPLE:

(+)Amethopterin + H_2O $\xrightarrow{\text{Carboxypeptidase G}}$ L-Glutamic Acid + 4-Amino-N^{10}-Methylpteroic Acid

CONDITIONS: T = 30°C, pH 7.3, A_{320nm}, 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:

	Test	Blank
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_{320nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

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

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

CALCULATION:

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

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.[1]
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 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^{10}-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.