

Enzymatic Assay of AMINOPEPTIDASE I

PRINCIPLE:



Abbreviation used:

AP I = Aminopeptidase I

CONDITIONS: T = 25°C, pH = 8.0, A_{405nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 50 mM Tris HCl Buffer with 100 mM Sodium Chloride and 1 mM Calcium Chloride, pH 8.0 at 25°C
(Prepare 100 ml in deionized water using Trizma Hydrochloride, Sigma Prod. No. T-3253, Sodium Chloride, Sigma Prod. No. S-9888, and Calcium Chloride, Dihydrate, Sigma Prod. No. C-3881. Adjust to pH 8.0 at 25°C with 1 M NaOH.)
- B. Methanol
(Use Methanol, Absolute, Sigma Stock No. 17-5.)
- C. 150 mM L-Leucine p-Nitroanilide Stock Solution
(Prepare 1 ml in Reagent B using L-Leucine p-Nitroanilide, Free Base, Sigma Prod. No. L-9125.)
- D. 3 mM L-Leucine p-Nitroanilide Solution (Leu-NA)
(Prepare 30 ml in Reagent A using Reagent C¹.)
- E. Aminopeptidase I Enzyme Solution
(Immediately before use, prepare a solution containing 0.1 - 0.5 unit/ml of Aminopeptidase I in cold Reagent A.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent D (Leu-NA)	2.95	2.95

Equilibrate to 25°C. Then add:

Reagent E (Enzyme Solution)	0.05	-----
Reagent A (Buffer)	-----	0.05

Immediately mix by inversion and record the increase in $A_{405\text{nm}}$ for approximately 5 minutes. Obtain the $\Delta A_{405\text{nm}}/\text{min}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{405\text{nm}}/\text{min Test} - \Delta A_{405\text{nm}}/\text{min Blank})(3)(\text{df})}{(10.6)(0.05)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

10.6 = Millimolar extinction coefficient of p-Nitroaniline
at $A_{405\text{nm}}$

0.05 = Volume (in milliliter) 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-leucine p-nitroanilide to L-leucine and p-nitroaniline per minute at pH 8.0, at 25°C, and 3.0 mM substrate concentration.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final assay concentrations are
49 mM Tris, 98 mM sodium chloride, 1 mM calcium chloride,
2% (v/v) methanol, 3 mM L-leucine p-nitroanilide, and
0.005 - 0.025 unit aminopeptidase.

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REFERENCE:

Spungin, A. and Blumberg, S. (1989) *European Journal of Biochemistry* **183**, 471-477

NOTES:

1. The substrate is close to its solubility limit in this buffer, so care must be taken to assure it is completely dissolved. Upon diluting Reagent C (Stock) with Reagent A (Buffer), mix vigorously using a vortex mixer.
2. This assay is based on the cited reference.
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.