Enzymatic Assay of AMINOPEPTIDASE
(EC 3.4.11.10)

PRINCIPLE:

L-Leucine p-Nitroanilide —AP—> L-Leucine + p Nitroaniline

Abbreviation used:
AP = Aminopeptidase

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

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 1 mM Tricine Solution
(Prepare 100 ml in deionized water using Tricine, Sigma Prod. No. T-0377.)

B. Methanol
(Use Methanol, Absolute, Sigma Stock No. 17-5.)

C. 50 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. 1.0 mM L-Leucine p-Nitroanilide Solution (Leu-NA)
(Prepare 5 ml by adding 0.1 ml of Reagent C to 4.9 ml of Reagent A.)

E. 200 mM Tricine Buffer, pH 8.0 at 25°C (Buffer)
(Prepare 100 ml in deionized water using Tricine, Sigma Prod. No. T-0377. Adjust to pH 8.0 at 25°C with 1 M NaOH.)

F. 20 mM Tricine Buffer with 0.05% (w/v) Bovine Serum Albumin, pH 8.0 at 25°C (Enz Dil)
(Prepare 25 ml in deionized water using Reagent E and Albumin, Bovine, Sigma Prod. No. A-4503. Adjust to pH 8.0 at 25°C with either 1 M HCl or 1 M NaOH if necessary.)

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REAGENTS: (continued)

G. Aminopeptidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.02 - 0.04 unit/ml of Aminopeptidase in cold Reagent F.)

PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container:

Reagent D (Leu-NA)	2.00
Reagent E (Buffer)	1.00
Deionized Water	7.00

Mix by swirling.

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

	Test	Blank
Reaction Cocktail	0.90	0.90

Equilibrate to 25°C. Then add:

Reagent G (Enzyme Solution)	0.10	------
Reagent F (Enz Dil)	------	0.10

Immediately mix by inversion and record the increase in ΔA_{405nm} for approximately 5 minutes. Obtain the ΔA_{405nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{405nm}/\text{min Test} - \Delta A_{405nm}/\text{min Blank})(1)(df)}{(10.8)(0.1)}$$

1 = Total volume (in milliliter) of assay
df = Dilution factor
10.8 = Millimolar extinction coefficient[1] of p-Nitroaniline at A_{405nm}
0.1 = Volume (in milliliter) of enzyme used

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

CALCULATIONS: (continued)

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

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.

FINAL ASSAY CONCENTRATIONS:

In a 1.00 ml reaction mix, the final assay concentrations are
20 mM Tricine, 0.4% (v/v) methanol, 0.18 mM L-leucine p-nitroanilide, 0.005% (w/v) bovine serum albumin, and 0.002 - 0.004 unit aminopeptidase.

REFERENCE:

Prescott, J.M. and Wilkes, S.H. (1976) *Methods in Enzymology*, XLV, Part B, 530-543

Spungin, A. and Blumberg, S. (1989) *European Journal Biochemistry* **183**, 471-777

NOTES:

1. The millimolar extinction coefficnet is described in Spungin A. and Blumberg, S. (1989).

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.