SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of LEUCINE AMINOPEPTIDASE, MICROSOMAL (EC 3.4.11.2)

PRINCIPLE:

$$\text{L-Leucine p-Nitroanilide} + H_2O \xrightarrow{\text{LAP}} \text{L-Leucine} + \text{p-Nitroaniline}$$

Abbreviation used:
LAP = Leucine Aminopeptidase, Microsomal

CONDITIONS: T = 37°C, pH = 7.2, A_{405nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 50 mM Sodium Phosphate Buffer, pH 7.2 at 37°C
(Prepare 100 ml in deionized water using Sodium Phosphate, Dibasic, Anhydrous, Sigma Prod. No. S-0876. Adjust to pH 7.2 at 37°C with 1 M HCl.)

B. Methanol
(Use Methanol, Sigma Prod. No. M-3641.)

C. 24 mM L-Leucine p-Nitroanilide Solution (LeuNA)
(Prepare 1 ml in Reagent B using L-Leucine p-Nitroanilide, Free Base, Sigma Prod. No. L-9125.)

D. Leucine Aminopeptidase, Microsomal Enzyme Solution
(Immediately before use, prepare a solution containing 0.10 - 0.15 unit/ml of Leucine Aminopeptidase, Microsomal in cold deionized water.)

PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	2.80	2.80
Reagent C (LeuNA)	0.10	0.10

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PROCEDURE: (continued)

Mix by inversion and equilibrate to 37°C. Monitor the A_{405nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

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

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

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{405nm}/\text{min Test} - \Delta A_{405nm}/\text{min Blank})(3)(df)}{(9.9)\ (0.1)}$$

3 = Total volume (in milliliters) of assay
df = Dilution factor
9.9 = Millimolar extinction coefficient[1] of p-Nitroaniline at 405 nm
0.1 = 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 7.2 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 47 mM sodium phosphate, 0.80 mM L-leucine p-nitroanilide, 3.3% (v/v) methanol and 0.01 - 0.015 unit leucine aminopeptidase, microsomal.

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REFERENCES:

Pfleiderer, G. (1970) *Methods in Enzymology*, XIX, 514-521

Lin, S.H. and Van Wart, H.E. (1982) *Biochemistry* **21**, 5528-5533

NOTES:

1. The millimolar extinction coefficient is described in Lin, S.H. and Van Wart, H.E. (1982).
2. This assay is based on the assay procedure described in Pfleiderer, G. (1982).
3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.