Enzymatic Assay of ALANINE AMINOPEPTIDASE
(EC 3.4.11.14)

PRINCIPLE:

$$\text{L-Alanine p-Nitroanilide} + H_2O \xrightarrow[Co^{++}]{\text{AAP}} \text{L-Alanine} + \text{p-Nitroaniline}$$

Abbreviation used:
AAP = Alanine Aminopeptidase

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

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 60 mM Potassium Phosphate Buffer, pH 7.2 at 37°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.2 at 37°C with 1 M KOH.)

B. 1.66 mM L-Alanine p-Nitroanilide Solution (L-Ala-NA)
(Prepare 30 ml in Reagent A using L-Alanine p-Nitroanilide, Hydrochloride, Sigma Prod. No. A-9325. **PREPARE FRESH**.)

C. 10 mM Tris HCl Buffer with 1 mM Cobalt Chloride, pH 8.0 at 37°C (Activation Buffer)
(Prepare 20 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.0 at 37°C with 1 M HCl and then add Cobalt Chloride, Hexahydrate, Sigma Prod. No. C-2644.[1])

D. Alanine Aminopeptidase Enzyme Solution, Non-Activated (Enz-Non Act)
(Immediately before use, prepare a solution containing 0.3 unit/ml of Alanine Aminopeptidase in cold deionized water.)

E. Alanine Aminopeptidase Enzyme Solution, Activated (Enz-Act)
(Immediately before use, prepare a solution containing 0.1 unit/ml of Alanine Aminopeptidase in cold Reagent C. Incubate at 37°C for 15 minutes to activate.)

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PROCEDURE:

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

	Nonactivated[1]		Activated[2]	
	Test	Blank	Test	Blank
Reagent B (L-Ala-NA)	3.00	3.00	3.00	3.00

Equilibrate to 37°C. Monitor the A_{405nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Deionized Water	------	0.10	------	------
Reagent C (Activation Buffer)	------	------	------	0.10
Reagent D (Enz Non Act)	0.10	------	------	------
Reagent E (Enz-Act)	------	------	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:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{405nm}/\text{min Test} - \Delta A_{405nm}/\text{min Blank})(3.1)(df)}{(9.62)(0.1)}$$

3.1 = Total volume (in milliliters) of assay
df = Dilution factor
9.62 = Millimolar extinction coefficient 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-alanine p-nitroanilide to L-alanine and p-nitroaniline per minute at pH 7.2 at 37°C.

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FINAL ASSAY CONCENTRATION:

Unactivated: In a 3.10 ml reaction mix, the final concentrations are 58 mM potassium phosphate, 1.61 mM L-alanine p-nitroanilide and 0.03 unit alanine aminopeptidase.

Activated: In a 3.10 ml reaction mix, the final concentrations are 58 mM potassium phosphate, 1.61 mM L-alanine p-nitroanilide, 0.32 mM Tris HCl, 0.03 mM cobalt chloride and 0.01 unit alanine aminopeptidase.

REFERENCES:

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

NOTES:

1. This solution should be clear and faint pink in color. If this solution is hazy or green/brown in color, it is not suitable for use.

2. The enzyme activity reported by Sigma is determined under conditions of the activated enzyme assay.

3. This assay is based on the cited reference.

4. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.