

Enzymatic Assay of PEPTIDASE¹

PRINCIPLE:

L-Leucine- β -Naphthylamide + H₂O $\xrightarrow{\text{Peptidase}}$ L-Leucine + β -Naphthylamine

β -Naphthylamine + NaNO₂ $\xrightarrow{\text{HCl}}$ Diazo Reagent

Diazo Reagent + N-(1-Naphthyl)ethylenediamine \rightarrow Blue Azo complex

CONDITIONS: T = 37°C, pH = 7.1, A_{580nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 100 mM Potassium Phosphate Buffer, pH 7.1 at 37°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.1 at 37°C with 1 M KOH.)
- B. 0.02% (w/v) L-Leucine β -Naphthylamide Solution (Leu-Nap)
(Prepare 50 ml in Reagent A using L-Leucine β -Naphthylamide Hydrochloride, Sigma Prod. No. L-0376, **or** use Leucine Aminopeptidase Substrate, Sigma Stock No. 251-1.)
- C. 2000 mM Hydrochloric Acid Solution (HCl)
(Prepare 100 ml in deionized water using Hydrochloric Acid, Sigma Prod. No. H-7020, or Sigma Stock No. 251-2.)
- D. 0.5% (w/v) Ammonium Sulfamate Solution (AS)
(Prepare 150 ml in deionized water using Sigma Prod. No. A-4630 or use Ammonium Sulfamate, 0.5% (w/v) Solution, Sigma Stock No. 251-3.)
- E. 0.2% (w/v) Sodium Nitrite Solution (Nit)
(Prepare 10 ml in deionized water using Sodium Nitrite, Sigma Prod. No. S-2252, or use Sodium Nitrite Tablets, Sigma Stock No. 251-4. **PREPARE FRESH.**)

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REAGENTS: (continued)

- F. 0.05% (w/v) N-(1-Naphthyl)ethylenediamine Solution (CLR)
(Prepare 100 ml in Reagent H using N-(1-Naphthyl)ethylenediamine Dihydrochloride, Sigma Prod. No. N-9125, **or** dissolve the contents of one bottle of N-(1-Naphthyl)ethylenediamine Dihydrochloride, Sigma Stock No. 251-5, in 110 ml of Ethanol (Reagent H).)
- G. 0.1257 mM β -Naphthylamine Solution (Std)
(Prepare 10 ml in deionized water using β -Naphthylamine, Sigma Prod. No. N-8381 **or** use Leucine Aminopeptidase (LAP) Calibration Solution, Sigma Stock No. 251-10.)
- H. 95% Ethanol (v/v)
(Use Ethanol, Nondenatured)
- I. Peptidase Enzyme Solution
(Immediately before use, prepare a solution containing 1 - 3 units/ml in cold Reagent A.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	-----	0.50
Reagent B (Leu-Nap)	0.50	0.50

Mix by swirling and equilibrate to 37°C. Then add:

Reagent I (Enzyme Solution)	0.50	-----
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Incubate for exactly 60 minutes. Then add:

Reagent C (HCl)	0.50	0.50
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COLORIMETRIC ASSAY:

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

	Test	Test <u>Blank</u>	Test <u>Std 1</u>	Test <u>Std 2</u>	Test <u>Std 3</u>	Test <u>Std 4</u>	Test <u>Std 5</u>	Test <u>Std Blank</u>
Test Soln	1.50	----	----	----	----	----	----	----
Blank Soln	----	1.50	----	----	----	----	----	----
Reagent C (HCl)	----	----	1.40	1.30	1.10	0.90	0.70	1.50
Reagent G (Std)	----	----	0.10	0.20	0.40	0.60	0.80	----

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COLORIMETRIC ASSAY: (continued)

Mix by swirling. Then add:

	<u>Test</u>	<u>Test Blank</u>	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Std 4</u>	<u>Std 5</u>	<u>Std Blank</u>
Reagent E (Nit)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

Mix by swirling and incubate at 25°C for 3 minutes. Then add:

Reagent D (AS)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
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Mix by swirling and incubate at 25°C for 3 minutes. Then add:

Reagent F (CLR)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
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Mix by swirling and incubate at 25°C for 45 minutes. Transfer solutions to suitable cuvettes and record the A_{580nm} for the Test, Blanks and Standards.

CALCULATIONS:

Standard Curve:

$$\Delta A_{580nm} \text{ Standard} = A_{580nm} \text{ Std} - A_{580nm} \text{ Std Blank}$$

Prepare a standard curve by plotting ΔA_{580nm} standard vs μmoles of β -Naphthylamine.

Sample Determination:

$$\Delta A_{580nm} \text{ Sample} = A_{580nm} \text{ Test} - A_{580nm} \text{ Blank}$$

Determine the μmoles of β -Naphthylamine liberated using the standard curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles of } \beta\text{-Naphthylamine liberated}) (df)}{(60) (0.5)}$$

df = Dilution factor

60 = Time of assay (in minutes) as per the unit definition.

0.5 = Volume (in milliliters) of enzyme used

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CALCULATIONS: (continued)

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

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

UNIT DEFINITION:

One unit will liberate 1.0 μ mole of β -naphthylamine from L-leucine β -naphthylamide per minute at pH 7.1 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 1.00 ml reaction mix, the final concentrations are 100 mM potassium phosphate, 0.01% (w/v) L-leucine β -naphthylamide and 0.5 - 1.5 units peptidase.

REFERENCE:

Martinek, R.G., Berger, L. and Broida, D. (1964) *Clin. Chem.* **10**, 1087.

NOTES:

1. Procedure can be replaced by using Leucine Aminopeptidase Kit, Sigma Stock No. 251-AW.
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.