

**Enzymatic Assay of PYROGLUTAMATE AMINOPEPTIDASE  
(EC 3.4.19.3)**

**PRINCIPLE:**

p Glu-β-Naphthylamide + H<sub>2</sub>O  $\xrightarrow{\text{PGLUA}}$  p Glu + β-Naphthylamine

β-Naphthylamine + NaNO<sub>2</sub>  $\xrightarrow{-\text{H}^+}$  Diazo Reagent

Diazo Reagent + N-(1-Naphthyl)-Ethylenediamine  $\longrightarrow$  Blue Azo Dye

Abbreviations:

p Glu = L-Pyroglutamic acid

PGLUA = Pyroglutamate Aminopeptidase

p Glu-β-Naphthylamide = L-Pyroglutamic acid β-Naphthylamide

**CONDITIONS:** T = 37°C, pH 8.0, A<sub>580nm</sub>, Light path = 1 cm

**METHOD:** Colorimetric

**REAGENTS:**

- A. 100 mM Potassium Phosphate Buffer with  
10 mM Ethylenediaminetetraacetic Acid,  
5.0% (v/v) Glycerol, and 5.0 mM Dithiothreitol,  
pH 8.0 at 37°C  
(Prepare 100 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate, Sigma Prod. No. P-5504, Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS, Glycerol, Sigma Prod. No. G-9012, and DL-Dithiothreitol, Sigma Prod. No. D-0632. Adjust to pH 8.0 at 37°C with 1 M HCl.)
- B. Methanol  
(Use Methanol, Absolute, Sigma Stock No. 17-5.)
- C. 22 mM L-Pyroglutamic Acid β-Naphthylamide Solution  
(p Glu-β-Nap)  
(Prepare 5 ml in Reagent B using L-Pyroglutamic Acid β-Naphthylamide, Sigma Prod. No. P-5891.)

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**REAGENTS:** (continued)

- D. 25% (w/v) Trichloroacetic Acid Solution (TCA)  
(Prepare 25 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution, approximately 100% (w/v), Sigma Stock No. 490-10.)
- E. 0.1% (w/v) Sodium Nitrite Solution (NaNO<sub>2</sub>)  
(Prepare 10 ml in deionized water using Sodium Nitrite, Sigma Stock No. 251-4.)
- F. 0.5% (w/v) Ammonium Sulfamate Solution (NH<sub>4</sub> Sulf)  
(Use Ammonium Sulfamate, 0.5% (w/v), Sigma Stock No. 251-3.)
- G. 95% (v/v) Ethanol  
(Prepare 125 ml in deionized water using Ethyl Alcohol, Denatured, Sigma Stock No. 27,074-1.)
- H. 0.05% (w/v) N-1-Naphthylethylenediamine Solution (NED)  
(Prepare by adding 110 ml of Reagent G to the contents of 1 bottle of N-1-Naphthylethylenediamine, Sigma Stock No. 251-5.)
- I. 0.0018% (w/v) β-Naphthylamine Standard Solution (Std)  
(Use LAP Calibration Solution, Sigma Stock No. 251-1.)
- J. Pyroglutamate Aminopeptidase Enzyme Solution  
(Immediately before use, prepare a solution containing 35 - 70 units/ml of Pyroglutamate Aminopeptidase in cold Reagent A.)

**PROCEDURE:**

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	1.00	1.00
Reagent C (p Glu-β-Nap)		0.10
		0.10

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

Reagent J (Enzyme Solution)	0.10	-----
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**PROCEDURE:** (continued)

Immediately mix by swirling and incubate at 37°C for exactly 15 minutes. Then add:

	<u>Test</u>	<u>Blank</u>
Reagent D (TCA)	1.00	1.00
Reagent J (Enzyme Solution)	-----	0.10

Mix by swirling.

**COLORIMETRIC ASSAY:**

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

	<u>Test</u>		<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Std 4</u>	<u>Std 5</u>	<u>Std</u>
	<u>Test</u>	<u>Blank</u>						<u>Blank</u>
Test Solution	1.00	----	----	----	----	----	----	----
Blank Solution	----	1.00	----	----	----	----	----	----
Reagent I (Std)	----	----	0.10	0.20	0.30	0.50	0.80	----
Deionized Water	----	----	0.90	0.80	0.70	0.50	0.20	1.00
Reagent E (NaNO <sub>2</sub> )	1.00	----	1.00	----	1.00	----	1.00	1.00
								1.00
								1.00
								1.00

Mix quickly by swirling and incubate at room temperature for 3 minutes. Then add:

Reagent F (NH <sub>4</sub> Sulf)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
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Mix quickly by swirling and incubate at room temperature for 3 minutes. Then add:

Reagent H (NED)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
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Mix quickly by swirling and incubate at room temperature for 45 minutes. Transfer the solutions to suitable cuvettes and record the A<sub>580nm</sub> for the Test, Test Blank, Standards, and Standard Blank using a suitable spectrophotometer.

**CALCULATIONS:**

Standard Curve:

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

Prepare a standard curve by plotting the  $\Delta A_{580\text{nm}}$  Standard vs nanomoles of  $\beta$ -Naphthylamine.

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**CALCULATIONS:** (continued)

Sample Determination:

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

Determine the nanomoles of  $\beta$ -Naphthylamine liberated using the standard curve.

$$\text{Units/ml enzyme} = \frac{(\text{nanomoles of } \beta\text{-Naphthylamine liberated})(2.2)(\text{df})}{(15)(0.1)}$$

2.2 = Total volume (in milliliters) of stopped reaction

df = Dilution factor

15 = Time (in minutes) of assay as per the Unit Definition

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 nanomole of L-pyroglutamic acid  $\beta$ -naphthylamide to L-pyroglutamic acid and  $\beta$ -naphthylamine per minute at pH 8.0 at 37°C.

**FINAL ASSAY CONCENTRATIONS:**

In a 1.20 ml reaction mix, the final concentrations are 92 mM potassium phosphate, 9.2 mM ethylenediaminetetraacetic acid, 4.6% (v/v) glycerol, 4.6 mM DL-dithiothreitol, 1.8 mM L-pyroglutamic acid  $\beta$ -naphthylamide, and 3.5 - 7.0 unit pyroglutamate aminopeptidase.

**REFERENCE:**

Szewczuk, A. and Mulczyk, M. (1969) *European J. Biochemistry* **8**, 63-67

**NOTES:**

1. This assay is based on the cited reference.

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**PROCEDURE:** (continued)

2. 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.**