

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

**PRINCIPLE:**

PGA-2-N Nap + Water  $\xrightarrow{\text{PGAP}}$  PGA + 2-N Nap

Abbreviations:

PGA-2-N Nap = L-Pyroglutamate- $\beta$ -Naphthyamide

PGA = L-Pyroglutamate

2-N Nap =  $\beta$ -Naphthylamine

**CONDITIONS:** T = 37°C, pH 8.00, A<sub>580nm</sub>, Light path = 1 cm

**METHOD:** Colorimetric

**REAGENTS:**

- A. 100 mM Potassium Phosphate with 10 mM EDTA, 5% Glycerol and 5 mM DTT, pH 8.00 at 37°C.  
(Prepare 100 ml in deionized water using Potassium Phosphate, Dibasic, Prod. No. P-5504, EDTA, Disodium, Stock No. ED2SS, Glycerol, Prod. No. G-7757 and DTT, Prod. No. D-0632. Adjust to pH 8.00 at 37°C with 2 N NaOH.)
- B. 20 mM L-Pyrrolidonyl- $\beta$ -Naphthylamide. (PNP)  
(Prepare 10 ml in Methanol, using L-Pyrrolidonyl- $\beta$ -Naphthylamide, Prod. No. P-5891.)
- C. 25% Trichloroacetic Acid.  
(Prepare 10 ml in deionized water using Trichloroacetic Acid, Prod. No. T-6399.)
- D. 0.2% Sodium Nitrite Solution (NaNO<sub>2</sub>)  
(Prepare 10 ml in deionized water using Sodium Nitrite, Stock No. 251-4.)
- E. 0.5% Ammonium Sulfamate Solution (Sulfamate)  
(Use Ammonium Sulfamate, Stock No. 251-3.)

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**REAGENTS:** (continued)

- F. N-1-Naphthylethylenediamine Solution (NED)  
(Prepare by adding 110 ml of 95% Ethyl Alcohol to contents of bottle of N-1-Naphthylethylenediamine, Stock No. 251-5.)
- G. LAP Calibration Solution  
(Use LAP Calibration Solution, Stock No. 251-10.)
- H. L-Pyroglutamate Aminopeptidase Enzyme Solution  
(Immediately before use, prepare a solution containing 10 mg solid/ml of L-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 B (PNP)	0.10	0.10

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

Reagent H (Enzyme Solution)	0.10	-----
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Immediately mix and incubate at 37°C for exactly 15 minutes. Then add:

Reagent C (TCA)	1.00	1.00
Reagent H (Enzyme Solution)	-----	0.10

Prepare standards and samples by pipetting (in milliliters) the following reagents into suitable containers:

	<u>Std1</u>	<u>Std2</u>	<u>Std3</u>	<u>Std4</u>	<u>Std</u> <u>Blank</u>	<u>Test</u>	<u>Test</u> <u>Blank</u>
Test	----	----	----	----	----	1.00	----
Blank	----	----	----	----	----	----	1.00
Reagent G (Std)	0.20	0.40	0.60	0.80	----	----	----
Deionized Water	0.80	0.60	0.40	0.20	1.00	----	----
Reagent D (NaNO <sub>2</sub> )	1.00	1.00	1.00	1.00	1.00	1.00	1.00

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**PROCEDURE:** (continued)

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

	<u>Std1</u>	<u>Std2</u>	<u>Std3</u>	<u>Std4</u>	<u>Std</u> <u>Blank</u>	<u>Test</u> <u>Test</u>	<u>Test</u> <u>Blank</u>
Reagent E (NH <sub>4</sub> Sulf)	1.00	1.00	1.00	1.00	1.00	1.00	1.00

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

Reagent F (NED)	2.00	2.00	2.00	2.00	2.00	2.00	2.00
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Mix quickly and incubate for 45 minutes at 25°C. Transfer the material into suitable cuvettes and record the A<sub>580</sub> for each using a suitable spectrophotometer.

**CALCULATIONS:**

Find the slope plotting the A<sub>580nm</sub> Standards vs LAP concentration. Use the slope (M) to determine the concentration of the test mixture.

$$\text{units/mg enzyme} = \frac{(A_{580\text{nm}} \text{ Test} - A_{580\text{nm}} \text{ Blank}) (2.2)}{(15) (M) (\text{mg protein/RM})}$$

2.2 = Volume of test Mixture

15 = Time of assay

RM = Reaction Mix (Total Volume = 1.2 ml)

**UNIT DEFINITION:**

One unit will hydrolyze 1.0 nanomole of L-pyroglutamic acid β-naphthylamide to L-pyroglutamic acid and β-naphthylamine per min at pH 8.0 at 37°C.

**FINAL ASSAY CONCENTRATION:**

In a 1.2 ml reaction mix, the final concentrations are 92 mM potassium phosphate, 9.2 mM EDTA, 4.6% glycerol, 4.6 mM DTT, 1.7 mM L-pyrrolidonyl-β-naphthylamide, 8.3% methanol and 1 mg solid L-pyroglutamate aminopeptidase.

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**NOTES:**

1. 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.**