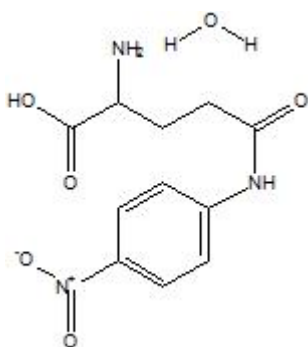


TECHNICAL INFORMATION

Catalog Number: 100663

gamma-L-Glutamyl-p-Nitroanilide, Hydrate

Structure:



Molecular Formula: C₁₁H₁₃N₃O₅·H₂O

Molecular Weight: 285.3

CAS #: 7300-59-6

Synonyms: L-gamma-Glu-pNA; GPNA; L-g-Glutamyl-p-nitroanilide

Physical Description: Off-white powder

Solubility: Soluble in 1 N HCl (50 mg/ml - clear, yellowish solution), 0.1 N NaOH (0.5% w/v - clear, yellowish solution)

Description: Suitable for use as a substrate for g-glutamyl transpeptidase.

Assay Procedure for the Determination of 4-Nitroaniline, bound, after acidic hydrolysis.

L-Glutamyl-4-nitroanilide + H₂O -----> L-glutamate + 4-nitroaniline

The increase in absorbance is measured at 405 nm.

Reagents:

1. Hydrochloric acid (min. 37%).
2. Phosphate buffer (0.1 mol/l; pH 7.0):
 - a. 1.36 g KH₂PO₄/100 ml double distilled water.
 - b. 2.28 g K₂HPO₄·3H₂O/100 ml double distilled water.
 - c. Adjust the pH of the 2b solution to 7.0 with the 2a solution.

Hydrolysis:

Exactly weigh 10-15 mg L-g-glutamyl-4-nitroanilide into a 25 ml volumetric flask, add 2 ml hydrochloric acid (1) and heat in a boiling water bath for 1 hour. Stopper the flask, cool to room temperature and adjust the volume to 25 ml with phosphate buffer (2) (= hydrolysate).

Wavelength: 405 nm; e₄₀₅ = 10.4 [mmol⁻¹ × 1 × cm⁻¹]

Light path: 1 cm
Temperature: 20-25°C

Total volume: 3.10 ml
Hydrolysate volume: 0.10 ml

Pipette into a cuvette:

buffer (2)	3.00 ml	read the absorbance A_1
hydrolysate	0.10 ml	mix, read the absorbance A_2

Calculation:

$$\Delta A = A_2 - A_1$$

$$\% \text{ Content of 4-nitroaniline}_{\text{total}} = \frac{3.10 \times (138/1000) \times 100}{\epsilon \times 0.10 \times 1 \times \text{sample weight [mg/ml hydrolysate]}} \times \Delta A$$

Assay for the Determination of 4-Nitroaniline, free

The absorbance is measured at 405 nm.

Reagents:

1. Phosphate buffer (0.1 mol/l; pH 7.0):

- 1.36 g KH_2PO_4 /100 ml double distilled water.
- 2.28 g $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ /100 ml double distilled water.
- Adjust the pH of the 1b solution to 7.0 with the 1a solution.

Sample:

Triturate in an agate mortar 25 mg gamma-L-Glutamyl-p-nitroanilide with some drops of double distilled water; transfer into a 25 ml volumetric flask with approximately 20 ml double distilled water; dissolve under heating at 50-60°C, cool at room temperature and adjust the volume to 25 ml with double distilled water.

Wavelength: 405 nm; $\epsilon_{405} = 10.4 [\text{mmol}^{-1} \times 1 \times \text{cm}^{-1}]$

Light path: 1 cm
Temperature: 20-25°C

Total volume: 6.0 ml
Sample volume: 3.0 ml

Pipette into test tubes:

	Blank	Sample
Buffer (1)		
	3.00 ml	3.00 ml
Double Distilled water		
	3.00 ml	--
Sample		
	--	3.00 ml

Mix, read the absorbance in the same cuvette.

Calculation:

$$\Delta A = A_{\text{sample}} - A_{\text{blank}}$$

$$\% \text{ 4-nitroaniline}_{\text{free}} = \frac{(138/1000) \times 2 \times 100}{\varepsilon \times l \times \text{sample weight [mg/ml]}} \times \Delta A$$

$$\text{Content of 4-nitroaniline}_{\text{total}} [\%] - \text{content of 4-nitroaniline}_{\text{free}} [\%] = \text{content of 4-nitroaniline}_{\text{bound}} [\%]$$

$$\% \text{ gamma-L-Glutamyl-4-nitroanilide} = \frac{\text{content of 4-nitroaniline}_{\text{bound}} [\%] \times 267.4}{138.1}$$

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