

Wako

Lysyl Endopeptidase[®] リシルエンドペプチダーゼ[®]

Lysyl Endopeptidase, originally isolated from the soil bacterium by Masaki *et al.*, cleaves specifically the peptides on the carboxy-terminal side of Lysine residues. This enzyme is very useful both in protein sequence analysis and in enzymatic synthesis of Lys-X compounds.

- Source** : Bacteria (See Notice.)
- Appearance** : Lyophilized form containing ca.10% Tris-HCl buffer, pH 8.0
- Activity** : Indicated on the label
- Molecular weight** : 27,000 (Gel filtration), 30,000 (SDS-PAGE)
- Solubility** : Soluble in water or buffer solutions.
- Stability** : Stable at 4°C for at least 24 hours, when dissolved in buffer of pH 4~11⁵⁾. Stable at 30°C in the range of pH 6~11, but unstable at 50°C or higher.
- Optimal pH** : 9.0~9.5 (Amidase activity)
- Isoelectric point** : 6.9~7.0
- Substrate specificity** :
Hydrolysable substrate..... Tos-Lys-OMe, Bz-Lys-NH₂,
Bz-Lys-pNA, Lys-pNA
Unhydrolysable substrate... Bz-Arg-NH₂, Bz-Arg-pNA,
Arg-pNA
- Inhibitors** : DFP, PMSF, TLCK

Assay method

1. Reagents

- A. 0.2 mol/L AMP buffer, pH 9.5
Dissolve 4.2 g of 2-Amino-2-methyl-1,3-propanediol in 150 mL of water, adjust to pH 9.5 with 1 mol/L HCl, and then add water to bring the volume up to 200 mL.

- B. 2.5 mmol/L Substrate solution
Dissolve 22.6 mg of *N*^α-Benzoyl-DL-lysine-*p*-nitroanilide Hydrobromide in 20 mL of water.
- C. 2 mmol/L Tris-HCl buffer, pH 8.0
Dissolve 0.24 g of 2-Amino-2-hydroxymethyl-1,3-propanediol in 900 mL of water, adjust to pH 8.0 with 1 mol/L HCl, and then add water to bring the volume up to 1 L.
- D. Enzyme solution
Dissolve 1 vial of Lysyl Endopeptidase in 1 mL of water added directly into the vial. In the case of 2AU vial, dispense 500 μL of the solution to a 25 mL flask, and then add Reagent C to bring the volume up to 25 mL.
- E. Stop Solution
Mix 55 mL of water and 45 mL of Acetic acid.

Trans^{*}

1. 試薬

- A. 0.2 mol/L AMP緩衝液、pH 9.5
2-アミノ-2-メチル-1,3-プロパンジオール 4.2 g を水 150 mL に溶解した後、1 mol/L 塩酸で pH 9.5 に調整し、水を加え 200 mL にする。
- B. 2.5 mmol/L 基質溶液
N^α-ベンゾイル-DL-リシン-*p*-ニトロアニリド臭化水素酸塩 22.6 mg を水 20 mL に溶解する。
- C. 2 mmol/L Tris-HCl 緩衝液、pH 8.0
2-アミノ-2-ヒドロキシメチル-1,3-プロパンジオール 0.24 g を水 900 mL に溶解した後、0.1 mol/L 塩酸で pH 8.0 に調整し、水を加え 1 L にする。
- D. 酵素溶液
本品 1 vial に全量ピペットを用いて水 1 mL を加え溶解させる。2AU 包装の場合、マイクロピペットを用いて、その溶液を 500 μL 取り、全量フラスコに入れ、C液を加え 25 mL にする。
- E. 反応停止液
水 55 mL と酢酸 45 mL を混ぜる。

2. Procedure

Reagent	Test	Blank
A	2.6 mL	2.6 mL
B	0.3 mL	0.3 mL
Pre-incubate at 30 °C for 5 minutes.		
D	0.1 mL	—
C	—	0.1 mL
Immediately mix, and incubate 30 °C for exactly 25 minutes		
E	1.0 mL	1.0 mL

Immediately, measure the absorbance at 405 nm of wavelength with water as the control.

Trans *

直ちに、波長 405 nm における吸光度を水を対照液として測定する。

3. Unit definition

One amidase unit (AU) is the amount of enzyme, which will produce 1 μ mol of *p*-nitroaniline per minute at 30 °C, pH 9.5.

(Calculation)

$$\text{AU/vial} = \frac{a - b}{25} \times \frac{1}{9.62} \times \frac{4.0}{0.1} \times c$$

a : Absorbance in test

b : Absorbance in blank

c : Dilution rate of Lysyl Endopeptidase

Trans *

3. 単位の定義

pH 9.5、30 °C で 1 分間に 1 μ mol の *p*-ニトロアニリンを生成する酵素量を 1AU とする。

(計算)

$$\text{AU/vial} = \frac{a - b}{25} \times \frac{1}{9.62} \times \frac{4.0}{0.1} \times c$$

a : 本試験の吸光度

b : 空試験の吸光度

c : 本品の希釈倍率

Notice

The source of this product had been indicated as “*Achromobacter lyticus*”, based on the physiological and morphological properties of the bacteria. However, by recent bacterial taxonomy, the bacteria were identified as *Lysobacter enzymogenes*.

Trans *

当製品について、菌発見当時の生理的・形態学的性質の見知から、由来は *Achromobacter lyticus* と表示させていただいておりましたが、近年の細菌分類学に基づいた再同定の結果、由来は *Lysobacter enzymogenes* であると結論いたしました。

〔Storage〕 Store at -20 °C.

〔Package〕

Code No.	Packaging
125-02543	2AU
129-02541	10AU

〔References〕

- 1) Masaki, T., Nakamura, K., Isono, M. and Soejima, M. : *Agr. Biol. Chem.*, **42**, 1443 (1978).
- 2) Morihara, K., Oka, T., Tsuzuki, H., Tochino, Y. and Kanaya, T. : *Biochem. Biophys. Res. Commun.*, **92**, 396 (1980).
- 3) Schwert, G. W. Takenaka, Y. : *Bioche. Biophys. Acta.*, **16**, 570 (1955).
- 4) Tuppy, H., Wissbauer, U. and Wintersberger, E. : *HoppeSeyler's Z. Physiol. Chem.*, **329**, 278 (1962).
- 5) Masaki, T., Tanabe, M., Nakamura, K. and Soejima, M. : *Biochem. Biophys. Acta.*, **660**, 44 (1981).
- 6) Masaki, T., Fujihashi, T., Nakamura, K. and Soejima, M. : *Biochem. Biophys. Acta.*, **660**, 51 (1981).

* : **Trans** is the Japanese translation.

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