〔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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0803Y03

Code No. **125 – 02543(2AU)**
Code No. **129 – 02541(10AU)**

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 fitration), 30,000 (SDS-PAGE)
Solubility	:Soluble in water or buffer solutions.
Stability	:Stable at 4℃, when dissolved in buffer of pH 5～12. Stable at 30℃ in the range of pH 6～11, but unstable at 50℃ 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-NH_2, Bz-Lys-*p*NA, Lys-*p*NA

Unhydrolysable substrate · · Bz-Arg-NH_2, Bz-Arg-*p*NA, Arg-*p*NA

Inhibitors :DFP, PMSF, TLCK

Assay method

1. Reagents

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

B. 2.5mmol/L Substrate solution
Dissolve 22.6mg of N^{α}-Benzoyl-DL-lysine-*p*-nitroanilide Hydrobromide in 20mL of water.

C. 2mmol/L Tris-HCl buffer, pH 8.0
Dissolve 0.24g of 2-Amino-2-hydroxymethyl-1,3-propanediol in 900mL of water, adjust to pH 8.0 with 1mol/L HCl, and then add water to bring the volume up to 1L.

D. Enzyme solution
Dissolve 1 vial of Lysyl Endopeptidase in 1mL of water added directly into the vial. In the case of 2AU vial, dispense 100μL of the solution to a 25mL flask, and then add Reagent C to bring the volume up to 25mL.

E. Stop Solution
Mix 55mL of water and 45mL of Acetic acid

Trans*

1. 試　薬

A. 0.2mol/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4.2gを水150mLで溶解させた後、1mol/L 塩酸でpH 9.5に調整し、水を加え200mLにする。

B. 2.5mmol/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 22.6mgを水20mLに溶解させる。

C. 2mmol/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 0.24gを水900mLに溶解させた後、0.1mol/L 塩酸でpH 8.0に調整し、水を加え1Lにする。

D. 酵素溶液
本品1vialに全量ピペットを用いて水1mL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100μL取り、全量フラスコに入れ、C液を加え20mLにする。

E. 反応停止液
水55mLと酢酸45mLを混ぜる。

2. Procedure

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

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

Trans*

直ちに、波長405nm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 μmol of *p*-nitroaniline per minute at 30℃, 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℃で1分間に1μmol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

Originally, the source of this product was indicated as "*Achromobacter lyticus*" based on the physiological and morphological properties of the bacteria. However, we confirmed that the 16S rDNA sequence was highly homologous to that of *Lysobacter*.

Trans*

当製品について、当初菌の生理的・形態学的性質から、由来は *Achromobacter lyticus* と表示させていただいておりましたが、16S rDNAの塩基配列を比較いたしましたところ、*Lysobacter*属と高いホモロジーが認められました。

〔Storage〕 Store at −20℃.

〔Package〕

125 − 02543	2AU
129 − 02541	10AU