Print

Endoproteinase Lys-C

Sequencing Grade

From *Lysobacter enzymogenes*

Cat. No. 11 420 429 001 5 µg
Cat. No. 11 047 825 001 3 × 5 µg

Version 20
Content version: April 2016

Store at +2 to +8°C

1. What this Product Does

Content

Lyophilizate

A film of humidity occasionally present in the vials can be due to the strong hygroscopic nature of the lyophilizate. Stability and function of the enzyme are not influenced. Endoproteinase Lys-C is isolated from *Lysobacter enzymogenes* as a highly purified and specific protease. The protease is suitable for the digestion of proteins in solution, in gels, and on blotting membranes.

Storage and Stability

Stable at +2 to +8°C until the expiration date printed on the label. The working solution of Endoproteinase Lys-C in double-distilled water may be used for a maximum of 1-2 days, when stored at +2 to +8°C.

Store dry!

Application

For protein-structure and sequence analysis. Suited for the digestion of proteins in polyacrylamide gels.

2. How to Use this Product

2.1 Before You Begin

General Handling Recommendations

The content of one vial may be used for several simultaneous digests. A new vial should be taken when repeating a digest in order to minimize the risk of contamination or autolysis.

2.2 Digestion of Proteins in Solution

Working Solution

Reconstitute lyophilized Endoproteinase Lys-C in 50 µl double-distilled water. This results in a buffer concentration of 50 mM Hepes, pH 8.0, 10 mM EDTA and 5 mg/ml raffinose. To avoid autolysis, the incubation temperature should not exceed +37°C.

Procedure

①	Dissolve the proteins to be sequenced in digestion buffer (25 mM Tris HCl, pH 8.5; 1 mM EDTA).
②	In the case of proteins that are hard to solubilize, add urea, SDS or guanidine HCl to the digestion buffer prior to solubilizing the protein. When applying urea we suggest that you also add 20 mM methylamine.
③	To achieve a suitable concentration of the denaturing agent in the digest, the protein solution has to be correspondingly diluted with buffer (Table 1).
④	The recommended amount of enzyme is 1/100 to 1/20 of the protein by weight.

Tab. 1: Activity determination of Endoproteinase Lys-C with Chromozym PL as substrate in the presence of stated concentrations of denaturing agents. Incubation of Endoproteinase Lys-C 200 µg/ml, with denaturing agent for 6 h at +25° C in 25 mM Tris-HCl buffer, pH 8.5; 1 mM EDTA.

Roche recommends also adding 20 mM methylamine when applying urea.

Denaturing agent	Concentration	Enzyme activity in %
without addition (control)	-	100
sodium dodecyl sulfate (SDS)	0.001% (w/v)	113
	0.01% (w/v)	136
	0.1% (w/v)	109
urea	0.1 M	122
	0.5 M	106
	1.0 M	90
	4.0 M	86
guanidine hydrochloride	0.1 M	60
	0.5 M	27
	1.0 M	12
acetonitrile	1% (v/v)	122
	5% (v/v)	157
	10% (v/v)	161

2.3 Digestion of Proteins in Gels or on Blotting Membranes

Procedure

Endoproteinase Lys-C can also be used for the "in gel" digestion of proteins (1, 2, 3). The reconstituted protease solution is further diluted with digestion buffer to 1-5 µg Endoproteinase Lys-C in 100 µl. Provide sufficient volume to the gel so that the gel is just covered or shrunken elements are reswollen.

Incubation Time

The incubation time should be chosen between 2 and 18 h at +37°C, depending on the amount of enzyme to be digested.

3. Additional Information on this Product

3.1 Product Characteristics

Molecular Weight

33 kDa (reduced)
30 kDa (not reduced)

Sequence of endoproteinase Lys-C

1 G V S G S C N I D V V C P E G N G H R D V I R S V A A Y S K
31 Q G T M W C T G S L V N N S A N D K K M Y F L T A N H C G M
61 T T A A I A S S M V V Y W N Y Q N S T C R A P G S S S S G A
91 N G D G S L A Q S Q T G A V V R A T N A A S D F T L L E L N
121 T A A N P A Y N L F W A G W D R R D Q N F A G A T A I H H P
151 N V A E K R I S H S T V A T E I S G Y N G A T G T S H L H V
181 F W Q A S G G V T E P G S S G S P I Y S P E K R V L G Q L H
211 G G P S S C S A T G A D R S D Y Y G R V F T S W T G G G T S
241 A T R L S D W L D A A G T G A Q F I D G L D S T G T P P V

3.2 Quality Control

Performance and purity are checked with HPLC.

Specificity and Nonspecificity Verification

Endoproteinase Lys-C is a serine protease that specifically cleaves peptide bonds C-terminally at lysine in Tris-HCl buffer, pH 7.0-9.0.

The specificity and nonspecificity of Endoproteinase Lys-C is verified using melittin as the substrate.

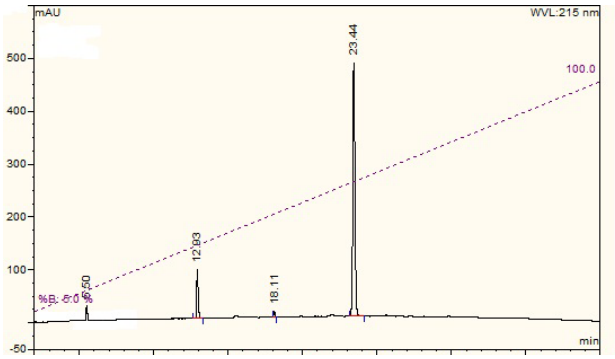

Fig. 1: Specificity of Endoproteinase Lys-C in reversed phase HPLC. High concentrations of Endoproteinase Lys-C (1 part by weight enzyme with 10 parts by weight melittin) are incubated for 1 h to detect the fragments of the specific digested substrate.

Digest	50 µg mellitin in 100 µl 25 mM Tris-HCl, pH 8.5; 1 mM EDTA + 5 µg Endoproteinase Lys-C in 50 µl water; 1 h at +37°C; reversed phase HPLC Injection volume: 50 µl
Column	Nucleosil 100-5-C18 4 x 100 mm, 5 µm
Solvent A	0.1% TFA (v/v) in water
Solvent B	0.1% TFA (v/v) in water; 70% acetonitrile (v/v)
Gradient	40 min linearly 0-100% B;
Flow rate	1 ml/min
Wavelength	215 nm
Fragments	5.50 min Arg(22)-Lys(23) and Arg(24)-Gln(26) 12.93 min Gly(1)-Lys(7), 23.44 min Val(8)-Lys(21

References

1 Kellner, R. (1995) *Biochemica* No. 2, Roche Applied Science.
2 Jenö, et al. (1995) *Anal. Biochem.* **224**, 75-82.
3 Shevchenko, A. et al. (1996) *Anal. Chem.* **68**, 850-858.
4 Eckerskorn, F. & Lottspeich, F. (1991) in: *Electrophorese Forum* (Radola, B-J-, Hrsg.) S. 283-288.
5 Fernandez, J. et al. (1994) *Anal. Biochem.* **21**, 112-117.

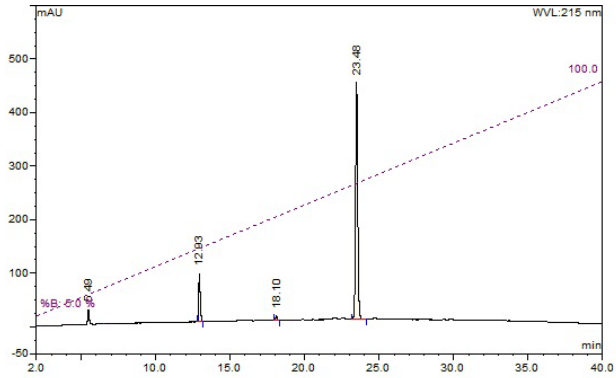

Fig. 2: Nonspecificity of Endoproteinase Lys-C in reversed phase HPLC. High concentrations of Endoproteinase Lys-C (1 part by weight enzyme with 10 parts by weight melittin) are incubated for 18 h to detect traces of impurities.

Digest	50 µg mellitin in 100 µl 25 mM Tris-HCl, pH 8.5; 1 mM EDTA + 5 µg Endoproteinase Lys-C sequencing grade in 50 µl water; 1 h at +37°C; reversed phase HPLC Injection volume: 50 µl
Column	Nucleosil 100-5-C18 4 x 100 mm, 5 µm
Solvent A	0.1% TFA (v/v) in water
Solvent B	0.1% TFA (v/v) in water; 70% acetonitrile (v/v)
Gradient	40 min linearly 0-100% B;
Flow rate	1 ml/min
Wavelength	215 nm
Fragments	5.50 min Arg(22)-Lys(23) and Arg(24)-Gln(26) 12.93 min Gly(1)-Lys(7), 23.44 min Val(8)-Lys(21

4. Supplementary Information

Changes to Previous Version

Editorial Changes

Text Conventions

To make information consistent and understandable, the following text conventions are used in this document:

Text Convention	Use
Numbered instructions labeled ①, ②, etc.	Stages in a process that usually occur in the order listed.

Symbols

Symbols are used in this document to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.

Ordering Information

Product	Pack Size	Cat. No.
Denaturation Reagents		
Guanidine thiocyanate	500 g	11 685 929 001
Sodium Dodecyl Sulfate	1 kg	11 667 289 001

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