

Endoproteinase Lys-C

Sequencing Grade

From *Lysobacter enzymogenes*

Cat. No. 11 420 429 001

5 µg

Version July 2007

Cat. No. 11 047 825 001

3 × 5 µg

Store at +2 to +8°C

1. What this Product Does

Contents

Lyophilizate

- ⊗ A film of humidity occasionally occurring in the lyophilization vials might be caused due to the strong hygroscopic nature of the lyophilizate. Stability and function of the enzyme are not influenced. Endoproteinase Lys-C sequencing grade is isolated from *Lysobacter enzymogenes* as a highly purified and specific protease. The protease is suitable to digest proteins in solution, in gels or on blotting membranes.

Storage and Stability

The lyophilizate is stable at +2 to +8°C until the expiration date printed on the label. A solution in double dist. water may be used for 1-2 days at maximum, if stored at +2 to +8°C.

- ⊗ The content of one vial may be used for several simultaneous digests. In order to repeat the digest a new vial should be used. Therefore, the utmost reproducibility can be guaranteed and probable contamination or autolysis will be avoided.

Product Characteristics

Specificity	Serine protease that specifically cleaves peptide bonds C-terminally at lysine in Tris-HCl buffer, pH 7.0-9.0.
Purity	The enzyme is free of impurities, which might interfere in the separation range of peptides in reversed phase HPLC (at highly sensitive detection at 206-230 nm). Function control by HPLC and purity control by SDS gel electrophoresis and silver staining ensure a constant quality with each lot.
Inhibitors	DFP, TLCK, Aprotinin, Leupeptin
Molecular weight	33 kD (reduced) 30 kD (not-reduced)

Application

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

2. How to Use this Product

Procedure

Lyophilized endoproteinase Lys-C Sequencing Grade is reconstituted in 50 µl double dist. water. This results in a buffer conc. of 50 mM Hepes, pH 8.0, 10 mM EDTA and 5 mg/ml raffinose. In order to avoid autolysis, the incubation temperature should not exceed 37°C. The proteins to be sequenced are dissolved in digestion buffer (25 mM Tris HCl, pH 8.5; 1 mM EDTA). In the case of proteins which are hard to solubilize, urea, SDS or guanidine hydrochloride should be added to the digestion buffer prior to solubilization of the protein. On application of urea it is recommended to also add 20 mM methylamine. In order to achieve a suitable concentration of the denaturing agent in the digest, the protein solution has to be correspondingly diluted with buffer (table). The recommended amount of enzyme is 1/100 to 1/20 of the protein by weight.

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. As much volume is given to the gel as every piece is just covered or shrunked pieces are reswollen.

The incubation time should be chosen between 2 and 18 hours at 37°C depending on the amount of enzyme.

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

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

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

Quality Control

The specificity of endoproteinase Lys-C sequencing grade is verified with melittin as substrate. High concentrations of endoproteinase Lys-C sequencing grade (1 part by weight enzyme with 10 parts by weight melittin) are incubated to detect traces of impurities e.g. contaminating proteases (see Fig.).

