

Product Information

Endoproteinase Lys-C from *Lysobacter enzymogenes* suitable for Protein Sequencing

Catalog Number **P3428**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

CAS RN 72561-05-8
EC 3.4.21.50
Synonym: Lys-C

Product Description

Endoproteinase Lys-C from *Lysobacter enzymogenes* is a serine endoprotease, which hydrolyzes peptide bonds at the carboxyl side of lysyl residues.¹⁻⁶ Lys-Pro and Lys-Glu bonds are also cleaved.⁵ Some minor non-specific cleavage has been reported.^{2,5} The protease readily cleaves at aminoethylcysteine residues.⁶

Endoproteinase Lys-C is HPLC purified, resulting in a product that is suitable for protein sequencing. In 100 mM NH₄HCO₃, pH 8.5, or 100 mM Tris HCl, pH 8.5, Lys-C specifically cleaves peptide bonds at the carboxyl side of lysine. It is widely used for protein sequencing work due to this highly specific cleavage of peptides resulting in a limited number of fragments.¹⁻⁶

Average molecular mass:¹ 27.96 kDa

Optimal pH:¹ ~8.5

Vial content: 5 µg of lyophilized Lys-C containing HEPES, EDTA, and raffinose.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Reconstitute the lyophilized product in 50 µl of water. The protease will be in a solution containing 50 mM HEPES, pH 8.0, 10 mM EDTA, and 5 mg/ml of raffinose.

Storage/Stability

Store the lyophilized powder desiccated at 2–8 °C. The hygroscopic nature of the lyophilized powder may make it appear wet. The activity and suitability of the enzyme is not affected.

After reconstitution in water, frozen aliquots can be stored for several weeks.⁴

Procedure

For peptide or protein digestion, a ratio between 1/20 and 1/100 (w/w) of enzyme to substrate is recommended.

1. Dissolve the peptide or protein to be digested in 100 mM NH₄HCO₃, pH 8.5, or 100 mM Tris HCl, pH 8.5.
2. Recommended incubation time is between 2 and 18 hours at 37 °C depending on the enzyme/substrate ratio.

Notes: Self-digestion of may occur if temperatures >37 °C are used.

Lys-C retains most of its activity in 2.0 M urea or 0.1% SDS.^{2,3,6}

A peptide such as melittin or the oxidized B chain of insulin should be run as a control for all experiments.

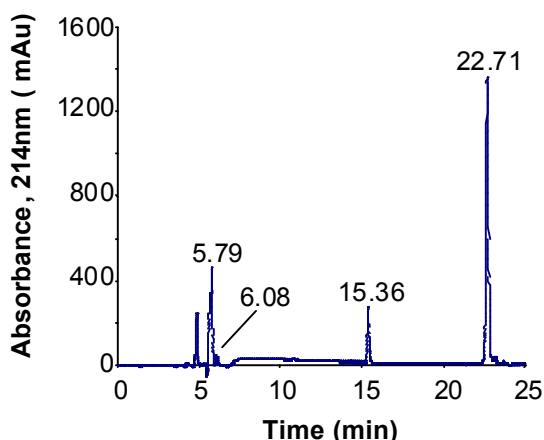
Endoproteinase Lys-C may also be used for in-gel digestions of proteins.⁷⁻¹¹

Results

The suitability of this product is demonstrated by the digestion of melittin (Catalog Number M4171; see Figure 1). The sequence of melittin is:

GIGAVLKVLTTGLPALISWIKRKRQQ

Figure 1.
Suitability Assay Of Lys-C



Melittin (100 µg) was digested with 5 µg of Lys-C for 18 hours at 37 °C in 100 µl of 100 mM NH₄HCO₃, pH 8.5. A 20 µg aliquot was separated on a Discovery[®] C₁₈ column (25 cm × 4.6 mm, 5 µm, Catalog Number 504971) using a 20 minute linear gradient from 5–50% B at 0.7 ml/min, and was detected in the UV at 214 nm and by mass spectrometry.

Solvent A: 0.1% (v/v) TFA in water

Solvent B: 0.08% (v/v) TFA in acetonitrile

The Lys-C peptide fragments were identified as follows:

Retention Time (min)	Mass (Da)	Fragment
5.79	429.3	Arg(24)-Gln(26)
6.08	302.3	Arg(22)-Lys(23)
15.36	656.4	Gly(1)-Lys(7)
22.71	1,512.0	Val(8)-Lys(21)

The two short hydrophilic peptides (retention times of 5.79 and 6.08) co-elute with the unbound buffer salts in the injection peak. The retention times for these two peptides were determined by searching for their expected masses.

During the 18 hour digestion only the expected peptides were generated with no indication of other major proteolytic activity. Under the experimental conditions the cleavage of the test peptide was complete in less than one hour.

References

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4. Stone, K.L. et al., *A Practical Guide to Protein and Peptide Purification for Microsequencing*, Academic Press, Inc. (New York, NY: 1989), p. 31.
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Related Products

HPLC Purified Products:

Product Name	Catalog Number
α-Chymotrypsin	C6423
Endoproteinase Arg-C	P6056
Endoproteinase Asp-N	P3303
Endoproteinase Glu-C	P6181
Endoproteinase Lys-C	P3428
Leucine aminopeptidase	L9776
Trypsin	T8658
Insulin Chain B, Oxidized	I1764
α-Melanocyte Stimulating Hormone	M4135

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