Endoproteinase Lys-C
Sequencing Grade
From Lysobacter enzymogenes

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.

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 +26°C in 25 mM Tris-HCl buffer, pH 8.5, 1 mM EDTA.

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

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 shrunk 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 GVGSQSNIDVCPEGNGHRDVIRSVAYSK
31 QGTMWCTGSLVNSSANDKKMYFLTANHCJM
61 TTAIAASSMVVYNNQNSTCRAPGSSSGSA
91 NGDGLAQQSTGAVRATNASSDFTLELN
121 TAAANAYLNFQAWDRDQNFAAGATAIHHP
151 NVKEKRIHSTDTVATEISGNGATGTSVLHV
181 FWSNQGVTPEGSQSGPSIEKRVQLH
211 GGPPSSCSATGADRDVYGRVFTSTWGGRTS
241 ATRLSDLMDAAGTQGIFIDGLDSTGTPP

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.

References
## Ordering Information

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<th>Product</th>
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<tr>
<td>Guanidine thiocyanate</td>
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<tr>
<td>Sodium Dodecyl Sulfate</td>
<td>1 kg</td>
<td>11 667 289 001</td>
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