

## Certificate of Analysis

### Endoproteinase Lys-C, Sequencing Grade

Part No.	Size
V107A	5µg

**Description:** Endoproteinase Lys-C, Sequencing Grade, specifically cleaves peptide bonds at the C-terminal of lysine.

**Biological Source:** *Lysobacter enzymogenes*.

**Molecular Weight:** 30kDa (33kDa when reduced).

**Form:** Lyophilized.

**Storage Conditions:** See the Product Information Label for storage temperature recommendations and expiration date.

**Usage Note:** Resuspend in 50µl of double-distilled water. This results in a storage buffer of 50mM HEPES (pH 8.0), 10mM EDTA and 5mg/ml raffinose. Resuspended Endoproteinase Lys-C, Sequencing Grade, can be used for up to 2 days when stored at 4°C.

Part# 9PIV107

Printed 3/09



## Quality Control Assays

This lot passes the following Quality Control specifications:

**Activity:** Determined by digestion of melittin substrate and HPLC analysis. After digestion for 1 hour at a ratio of 1:200 or 1:20 protease:substrate, all substrate is consumed and the same lysine-specific peaks are seen with both amounts of protease.

**Specificity:** Using melittin as a substrate, a comparison of digestion products at 18 hours of incubation at 37°C and digestion products at 1 hour of incubation shows ≤5% nonspecific cleavage by HPLC analysis.

**Purity:** Greater than or equal to 90% pure by SDS-PAGE analysis.



# Promega

#### Promega Corporation

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Signed by:

J. Stevens, Quality Assurance

Part# 9PIV107  
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## I. Protocol

### Preparation of Protein

In general proteins require efficient solubilization, denaturation and disulphide bond reduction (with subsequent alkylation) for optimal digestion and more complete sequence coverage. The following optional steps are provided as a guideline to facilitate protease digestion with this product.

- Solubilization/Denaturation:** Dissolve protein in 25mM Tris-HCl, 1mM EDTA (pH 8.5). Proteins that are difficult to dissolve or require denaturation for efficient digestion can be solubilized in a minimum volume in a denaturant such as 6–8M urea or 6M guanidine HCl at room temperature to 37°C for up to one hour. For some proteins, it may be beneficial to heat the sample to 60°C over this time period (95°C for 15–20 minutes for extreme cases). ProteaseMAX™ Surfactant can be used (0.01–0.2%) in 25mM Tris-HCl, 1mM EDTA (pH 8.5) in a minimum volume and does not require heating to be effective.
- Disulphide Reduction:** To the dissolved protein add DTT (or β-mercaptoethanol) to a final concentration of 5mM; heat this sample at 50–60°C for 20 minutes.
- Alkylation:** Allow the reduced protein mixture to cool to room temperature, and add iodoacetamide to a final concentration of 15mM. Incubate in the dark for 15 minutes at room temperature.
- Finally adjust the reaction volume with 25mM Tris-HCl, 1mM EDTA (pH 8.5) such that the urea concentration is 1M or less, the guanidine concentration is 0.1M or less, or the ProteaseMAX™ Surfactant concentration is at or below 0.025%.

### Enzyme Reconstitution

This product is lyophilized in the presence of HEPES, EDTA and raffinose. Dissolving the product in 50µl of double-distilled water will result in an optimal buffer concentration of 50mM HEPES, 10 mM EDTA (pH 8.0) and 5mg/ml raffinose.

### Digestion

Add Endoproteinase Lys-C, Sequencing Grade, to a final protease:protein ratio of 1:100 to 1:20 (w/w) and incubate sample for 2–18 hours at 37°C. The reaction may be stopped, if desired, by adding 0.5% trifluoroacetic acid.

**Note:** The presence of up to 0.1M guanidine or up to 1M urea in the digestion may reduce the activity of Endoproteinase Lys-C, Sequencing Grade, by up to 40% and 10%, respectively. The addition of ProteaseMAX™ Surfactant up to the recommended amount will not reduce the activity of Endoproteinase Lys-C, Sequencing Grade.

## II. Related Products

Product	Size	Cat. #
ProteaseMAX™ Surfactant, Trypsin Enhancer	1mg	V2071
	5 × 1mg	V2072
Trypsin Gold, Mass Spectrometry Grade	100µg	V5280
Sequencing Grade Modified Trypsin	100µg	V5111
Sequencing Grade Modified Trypsin, Frozen	100µg	V5113
Chymotrypsin, Sequencing Grade	25µg	V1061
	4 × 25µg	V1062