

## Certificate of Analysis

### rLys-C, Mass Spec Grade

Part No.	Name	Size
V167A	rLys-C, Mass Spec Grade	15µg
V181A	Resuspension Buffer	500µl

**Description:** rLys-C, Mass Spec Grade, specifically cleaves peptide bonds at the C-terminal of lysine. Recommended for use in solutions with up to 8M Urea.

**Biological Source:** *Escherichia coli*.

**Molecular Weight:** 27.7kDa.

**Form:** Lyophilized.

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

**Usage Note:** Resuspend in desired volume of Resuspension Buffer. Resuspended rLys-C, Mass Spec Grade, can be stored up to one month at -20°C.

Part# 9PIV167

Revised 2/20



AF9PIV167 0220V167

## Quality Control Assays

This lot passes the following Quality Control specifications:

**Activity:** Determined by digestion of a peptide 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 a substrate, a comparison of digestion products at 18 hours incubation at 37°C and digestion products at 1 hour incubation at 37°C shows ≤10% nonspecific cleavage by HPLC analysis.

**Purity:** Greater than or equal to 95% pure by HPLC analysis.

**Mass Spec Analysis:** Horse myoglobin is dissolved in 6M urea and denatured for 1 hour at 37°C. The denatured myoglobin is diluted in 25mM Tris, 1mM EDTA (pH 8.5) (final urea concentration in reaction is 0.4M), and incubated with rLys-C at 37°C. After an 18-hour incubation, the digest is analyzed by MALDI or LC/MS. The digest must yield at least 5 peptides with monoisotopic masses that match the peptides generated in a theoretical digest of myoglobin by Lys-C.

### Usage Information on Back



# Promega

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Part# 9PIV167  
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Signed by:

R. Wheeler, Quality Assurance



## 1. Amino Acid Sequence

### Sequence origin: *Pseudomonas aeruginosa* Lys-C

AGYRDGFGASGSCEVDVAVCATQSGTRAYDNATAAVAKMVFSSADGGSYICTGTLNNGN-  
SPKRQLFWSAAHCIEDQATAATLQTIWFYNTTQCYGDASTINQSVTLTGGANILHRDAKRD-  
TLLELKRTPPAGVFYQGW SATPIANGSLGHDIIHPRGDAAKKYSQGNVSAVGVTYDGHTAL-  
TRVDWPSAVVEGGSSGSLLTVAGDGSYQLRGGLYGGPSYCGAPTSQRNDYFSDFGSVYS-  
QISRYFAPHQHQQHQHQ

## 2. 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 8M urea or ProteaseMAX™ Surfactant (0.01–0.2%) in 25mM Tris-HCl, 1mM EDTA (pH 8.5).
- Disulphide Reduction:** To the dissolved protein, add DTT (or β-mercaptoethanol) to a final concentration of 5mM and incubate at room temperature for 30 minutes.
- Alkylation:** Add iodoacetamide to a final concentration of 10mM. Incubate in the dark at room temperature for 30 minutes.

**Note:** If ProteaseMAX™ Surfactant was used to solubilize protein, adjust the reaction volume with 25mM Tris-HCl, 1mM EDTA (pH 8.5) such that the surfactant concentration is at or below 0.025%. Adding ProteaseMAX™ Surfactant up to the recommended amount will not reduce the activity of rLys-C, Mass Spec Grade.

### Enzyme Reconstitution

This product is lyophilized in the presence of trehalose (0.5mg per vial). Dissolving in 75µl of Resuspension Buffer will result in the solution containing 0.2µg/µl rLys-C, Mass Spec Grade, 50mM acetic acid and 6.7mg/ml trehalose.

### Digestion

Add rLys-C, Mass Spec Grade, to a final protease:protein ratio of 1:50 to 1:20 (w/w) and incubate sample for 2–18 hours at 37°C. The reaction may be stopped, if desired, by adding trifluoroacetic acid to a final concentration of 0.5–1%.

## 3. Related Products

Product	Size	Conc.	Cat.#
Asp-N, Sequencing Grade	2µg		V1621
Arg-C, Sequencing Grade	10µg		V1881
Chymotrypsin, Sequencing Grade	25µg		V1061
	100µg (4 × 25µg)		V1062
Elastase	5mg		V1891
Endo H	10,000u	500u/µl	V4871
	50,000u	500u/µl	V4875
Fetuin	500µg	10mg/ml	V4961
Glu-C, Sequencing Grade	50µg (5 × 10µg)		V1651
Immobilized Trypsin	2ml		V9012
	4ml (2 × 2ml)		V9013
Pepsin	250mg		V1959
PNGase F	500u	10u/µl	V4831
ProteaseMAX™ Surfactant, Trypsin Enhancer	1mg		V2071
	5 × 1mg		V2072
Protein Deglycosylation Mix	20 reactions		V4931
Sequencing Grade Modified Trypsin	100µg (5 × 20µg)		V5111
Sequencing Grade Modified Trypsin, Frozen	100µg (5 × 20µg)		V5113
Thermolysin	25mg		V4001
Trypsin Gold, Mass Spectrometry Grade	100µg		V5280
Trypsin/Lys-C Mix, Mass Spec Grade	20µg		V5071
	100µg		V5072
	100µg (5 × 20µg)		V5073