

## Certificate of Analysis

### Lys-N, Mass Spec Grade:

Part No.	Size
VA118A	20µg

**Description:** Lys-N, Mass Spec Grade (Cat.# VA1180), specifically cleaves peptide bonds at the N-terminal side of lysine residues.

**Expiration Date:** See the Product Information Label for expiration date.

**Form:** Lyophilized.

**Storage Conditions:** Store the lyophilized product at  $-30^{\circ}\text{C}$  to  $-10^{\circ}\text{C}$ .

**Usage Note:** Reconstitute in desired volume of ultrapure water and mix gently. Reconstituted Lys-N, Mass Spec Grade, can be stored up to one month at  $-20^{\circ}\text{C}$ . Avoid freeze-thaw cycles.

Part# 9PIVA118

Printed 3/18



AF9PIVA1180318VA118

## Quality Control Assays

This lot passes the following Quality Control specification:

**Mass Spec Analysis:** Denatured horse myoglobin is incubated with 47mM Tris (pH 8.0) and Lys-N at  $37^{\circ}\text{C}$  for 18 hours. The digest is analyzed by LC/MS. At least five fragments from the myoglobin digest must match the expected myoglobin digest fragments. Fragments from the digest must be  $\pm 1$  AMU of the expected mass.

### Usage Information on Back



## Promega

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Signed by:

R. Wheeler, Quality Assurance

Part# 9PIVA118  
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## Before You Begin

The following protocols serve only as guidelines, since digestion conditions vary depending on the objective of the experiment. Typical variables to optimize include:

- Enzyme-to-substrate ratio: 1:20 to 1:100.
- Digestion time: 1–18 hours. Increased proteolysis at glutamic acid residues may be observed as digestion times increase.
- Buffer pH: Lys-N, Mass Spec Grade, has maximal activity at pH 8, but pH buffers ranging from 7–9 can be used. Increase digestion times accordingly.
- Denaturants: Lys-N is active in up to 6M urea, although activity will vary depending on concentration.
- Reducing agent and alkylating agent concentrations, as well as testing alternate reagents such as DTT and iodoacetic acid.

**Note:** Avoid the use of EDTA, as the required Zn(II) cofactor may be removed from the protein active site.

## Digestion of Complex Mixtures

This protocol is an example of the use of Lys-N, Mass Spec Grade (Cat.# VA1180), to digest a complex protein mixture such as yeast or human extract.

1. Reconstitute Lys-N, Mass Spec Grade, by adding 40µl of water and mixing gently. Store on ice or at 4°C. Single-use aliquots can be prepared and stored at –80°C.
2. Thaw one vial of yeast or human extract (Cat.# V7341 or Cat.# V6941; 1mg at 10µg/µl in 6.5M urea). Reduce by addition of 1µl of 0.1M TCEP (final concentration of 1mM). Incubate at 37°C for 60 minutes.
 

**Note:** Avoid temperatures higher than 37°C due to the presence of urea. Higher temperatures can cause carbamylation.
3. Alkylate by adding 1.0µl of 0.3M iodoacetamide (IAM; final concentration of 3mM) and incubate in the dark at room temperature for 30 minutes.
4. To a digestion tube add:
  - a. 10µl (100µg) of yeast or human extract.
  - b. 17µl of 8M urea, 50mM Tris (pH 8; 6M final concentration). This will bring the final concentration to 2M (taking into account the amount of urea from the original samples, 6.5M).
  - c. 73µl of 50mM Tris (pH 8).
5. Initiate digestion by adding 2.0µg (4.0µl) of Lys-N for a final enzyme:substrate ratio of 1:50.
6. Incubate at 37°C for 2–18 hours.
7. Terminate the reaction by acidification with TFA or formic acid (typically ~0.1–1% final concentration or until a pH of ~2–3 is reached).
8. Prior to LC-MS analysis, desalt the digested peptides either with offline SPE or with an online C<sub>18</sub> trap column.

## Digestion of Individual Proteins

This protocol is an example of the use of Lys-N, Mass Spec Grade, to digest proteins such as IgG that may be resistant to proteolytic digestion unless fully denatured, reduced and alkylated.

1. Reconstitute Lys-N, Mass Spec Grade, by adding 40µl of water and mixing gently. Store on ice or at 4°C.
2. Denature purified protein in 8M urea, 50mM Tris (pH 8) as follows:
  - a. Prepare purified protein stock at a concentration of 10mg/ml.
  - b. Dilute the protein to 0.4–2mg/ml in 8M urea, 50mM Tris (pH 8) in a volume of 100µl.
  - c. Mix and incubate for 30 minutes at 37°C.
3. Reduce by adding TCEP to 2mM and incubating for 60 minutes at 37°C.
4. Alkylate by adding iodoacetamide (IAM) to 4mM and incubating for 30 minutes at room temperature in the dark. For example, add 6.5µl of 0.5M IAM.
5. Dilute protein 4-fold (to less than 2M urea). For example, if your protein concentration is 2mg/ml in a volume of 100µl, add 300µl of 50mM Tris (pH 8.0). The final protein concentration will be 0.5mg/ml, and the urea concentration will be 2M.
6. Add Lys-N, Mass Spec Grade, at the desired enzyme:substrate ratio (E/S; 1:20–1:100) and incubate at 37°C for 1–18 hours. For example, if your protein concentration is 0.5mg/ml in a volume of 400µl, and you want to add enzyme at 1:50 E/S, add 8µl of 0.5mg/ml Lys-N (see **Before You Begin** above regarding optimization).
7. Terminate the reaction by acidification using TFA or formic acid (typically 0.1–1% final concentration or until a pH of ~2–3 is reached) and proceed to LC-MS.

## Related Products

Product	Size	Conc.	Cat.#
Lys-C, Mass Spec Grade	20µg		VA1170
rAsp-N, Mass Spec Grade	10µg		VA1160
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
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
rLys-C, Mass Spec Grade	15µg		V1671
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
IdeS Protease (lyophilized)	5,000 units		V7511
	25,000 units (5 × 5,000 units)		V7515
IdeZ Protease (lyophilized)	5,000 units		V8341
	25,000 units (5 × 5,000 units)		V8345