Certificate of Analysis

Lys-C, Mass Spec Grade:

Part No.	Size
VA117A	20µg

Description: Lys-C, Mass Spec Grade (Cat.# VA1170), specifically cleaves peptide bonds at the C-terminal end of lysine residues.

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

Form: Lyophilized.

Storage Conditions: Store the lyophilized product at –30°C to –10°C.

Usage Note: Reconstitute in desired volume of ultrapure water and mix gently. Reconstituted Lys-C, Mass Spec Grade, can be stored up to one month at –20°C. Avoid freeze-thaw cycles.

Part# 9PIVA117

Printed 3/18

AF9PIVA1170318VA117

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-C at 37°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 ±1 AMU of the expected mass.

Usage Information on Back

Signed by:

R. Wheeler, Quality Assurance

Promega

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Usage Information

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-C, 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-C 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.

Digestion of Complex Mixtures

This protocol is an example of the use of Lys-C, Mass Spec Grade (Cat.# VA1170), to digest a complex protein mixture, such as yeast or human extract.

1. Reconstitute Lys-C, 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). 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-C 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_{18} trap column.

Digestion of Individual Proteins

This protocol is an example of the use of Lys-C, 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-C, 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.
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-C, 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-C (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-N, Mass Spec Grade	20µg		VA1180
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

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