Mass Spectrometry-Grade Endoproteinases

Catalog Numbers 90051, 90053, 90054, 90056, 90307

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Thermo Scientific™ Mass Spectrometry-Grade Endoproteinases offer specific cleavage at multiple sites that enable exploration of primary protein structure (see Table 1). Effective protein characterization and identification by mass spectrometry (MS) begins with protein digestion. Trypsin is the protease of choice for accomplishing this task; however, digestion with alternative proteases, such as Glu-C, Lys-C, Asp-N, or chymotrypsin, can further improve sequence coverage (Lahm and Langen, 2000).

Table 1 Cleavage sites for the Thermo Scientific™ Mass Spectrometry-Grade Endoproteinases.

Protease	Cleavage specificity
Chymotrypsin	Carboxyl side of tyrosine, phenylalanine, tryptophan, and leucine residues
Lys-C	Carboxyl side of lysine residues
Glu-C	Carboxyl side of glutamic acid
Asp-N	Amino side of aspartic acid residues

Comprehensive protein characterization is facilitated by increased sequence coverage from overlapping peptides and improved peptide chromatographic separation, ionization, or fragmentation. For example, Lys-C cleavage after lysine residues generates larger peptides than those generated by trypsin alone, and chymotrypsin cleavage of hydrophobic regions provides complementary peptides to trypsin. These larger or more hydrophobic peptides interact more strongly with reverse phase columns and can improve detection of peptides with hydrophilic modifications, including phosphorylation or glycosylation. The masses of these larger peptides can be accurately measured at higher charge states on high-resolution mass spectrometers, reducing the database search time and increasing the sequence coverage and confidence. Finally, larger peptides can be fragmented effectively by MS fragmentation techniques like electron transfer dissociation (ETD), leaving post-translational modifications intact (Coon et al., 2005).

Contents and storage

Item	Cat. No.	Amount	Storage
Lys-C Endoproteinase, MS Grade	90051	20 µg	–20°C
	90307	100 µg	
Asp-N Endoproteinase, MS Grade	90053	2 µg	
Glu-C Endoproteinase, MS Grade	90054	5 x 10 µg	
Chymotrypsin Endoproteinase, TLCK treated, MS Grade	90056	4 x 25 µg	

Additional information

- Reduction and alkylation of cystine residues using dithiothreitol (DTT) and iodoacetamide, respectively, will minimize the appearance of unknown masses from disulfide bond formation and side-chain modification, improving detection of cysteine-containing peptides. Alkylation with iodoacetamide increases the mass of a peptide by 57.02 Da for each cysteine present.

- Chymotrypsin is treated with N-alpha-p-tosyl-L-lysine chloromethyl ketone (TLCK) to eliminate residual trypsin interference. Chymotrypsin activity is improved by adding 10 mM calcium chloride (Galvani et al., 2001).
- Lys-C is resistant to chemical denaturation and is active at pH 8.0. Lys-C is uniquely more active in highly basic environments (pH 9.5). Lys-C digestions are compatible with up to 8 M urea, which improves sequence coverage.
- Glu-C activity and cleavage specificity is affected by buffer conditions. Glu-C has maximal activity at either pH 4.0 or pH 8.0. In ammonium biocarbonate and other non-phosphate buffers, Glu-C cleaves on the C-terminal side of glutamic acid. Glu-C cleaves on the C-terminal side of both glutamic acid and aspartic acid residues in phosphate buffer.
- Asp-N is a metalloproteases and requires small quantities of zinc to enhance activity (Drapeau et al., 1972; Tarentino, 2004).

Required materials not supplied

- Urea (Cat. No. 29700)
- Ammonium bicarbonate (Cat. No. 370930250)
- DTT (Dithiothreitol, Cat. No. 20290 or A39255)
- Iodoacetamide (Cat. No. A39271)
- Calcium chloride (Cat. No. C79-500)
- Hydrochloric acid (Cat. No. 24308)
- Zinc acetate (Cat. No. 370080250)
- Tris-HCl (Cat. No. AM9855G)
- High purity/MS grade water (Cat. No. 51140)

Before you begin

1 Prepare reducing reagent

1. Dissolve 7.7 mg of dithiothreitol (DTT) in 100 µL of ultrapure water for a final concentration of 500 mM.
2. Transfer the solution to a labeled microcentrifuge tube.
3. Store aliquots of the reducing reagent at –20°C.

2 Prepare alkylating reagent

1. Immediately before use, dissolve 9.3 mg of iodoacetimide (IAA, Cat. No. A39271) in 100 µL of ultrapure water for a final concentration of 500 mM in an amber or foil-wrapped microcentrifuge tube.
2. Discard any remaining IAA solution after use.

3 Prepare digestion buffer and reconstitute enzyme

1. Prepare the appropriate digestion buffer for the specific protease (see Table 2).

   Typically, 5X ammonium bicarbonate (500 mM, pH 8.0) buffer is diluted with the protein sample and water to the final volume.
2. Prepare the appropriate enzyme reconstitution solution for the specific protease (see Table 2).

3 Prepare digestion buffer and reconstitute enzyme *(continued)*

3. Dissolve the protease in a sufficient volume of the enzyme reconstitution solution to yield a 1 mg/mL final concentration.

   A typical final protease-to-protein substrate ratio is 1:20.

4. Aliquot the remaining reconstituted enzyme in single-use volumes and store at –80°C.

Table 2 Enzyme-specific reconstitution solutions and digestion buffers.

Enzyme	Enzyme reconstitution solution	5X digestion buffer
Chymotrypsin	1 mM HCl	500 mM Tris-HCl (pH 8.0), 10 mM calcium chloride
Lys-C	Ultrapure water	500 mM ammonium bicarbonate (pH 8.0) or 2 M urea
Glu-C	Ultrapure water	500 mM ammonium bicarbonate (pH 8.0) or 500 mM ammonium acetate (pH 4.0)
Asp-N	Ultrapure water	250 mM Tris-HCl with 2.5 mM zinc acetate (pH 8.0)

Perform protein digestion

1 Reduce and alkylate proteins

1. Using a 0.5–0.7 mL microcentrifuge tube, add 10 µL of protein (1–50 µg), 29 µL of ultrapure water, and 10 µL of 5X digestion buffer.
2. *(Optional)* Verify that the pH of the solution is approximately 8.0 (or pH 4.0 for Glu-C) using pH paper.
3. Add 1 µL of 500 mM DTT for a final concentration of 10 mM and mix. Incubate at 60°C for 45 minutes.
4. Cool to room temperature.
5. Add 3.2 µL of freshly prepared 500 mM IAA to the reduced protein sample for a final concentration of 30 mM (see “Prepare alkylating reagent” on page 2). Vortex and centrifuge briefly. Discard any remaining reconstituted IAA.
6. Incubate the reaction at room temperature for 30 minutes protected from light.
7. Add 1 µL of 500 mM DTT to quench the alkylation reaction.

2 Digest proteins

1. Add 0.5–2.5 µL of 1 mg/mL protease for a final 1:20 enzyme-to-protein sample ratio. Mix the reaction.
2. Incubate the tube at 37°C for 16–24 hours (overnight).
3. Centrifuge the tube briefly to collect all liquid in the base of the tube.
4. Store samples at –80°C until mass spectrometric analysis. Before MS analysis, acidify and clean up samples with C18 spin columns (Pierce™ C18 Spin Tips, Cat. No. 84850) or equivalent.

Troubleshooting

Observation	Possible cause	Recommended action
No digestion	Incorrect pH or buffer conditions.	Use the specific buffer as listed in Table 2 and verify that the pH is correct.
	Reduced enzymatic activity.	Reconstitute enzyme immediately before use and make single-use aliquots to avoid multiple freeze/thaw cycles.
Precipitation after alkylation	Too much reduction/alkylation buffer for quantity of protein being digested.	Quench alkylation reaction using 10 mM DTT.
Incomplete sequence coverage	Incomplete digestion.	Reconstitute enzyme immediately before use and use the appropriate digestion buffer.
	Too few, too many, or unevenly distributed protease digestion sites.	Digest sample with multiple proteases separately and combine results (e.g., multiconsensus reports in Thermo Scientific™ Proteome Discoverer™ Software).
Over-alkylation	Alkylation was allowed to proceed for too long.	Alkylate at room temperature for 30 minutes and quench reaction with 10 mM DTT.
Incomplete alkylation or incomplete recovery of alkylated peptides	Used old or inactive iodoacetamide solution.	Prepare iodoacetamide solution immediately before use, and protect it from light.
Too much background noise on MS	Buffers, salt, or urea interference.	Clean up sample before analysis with reversed-phase tips or spin cartridges (i.e., Pierce™ Peptide Desalting Spin Columns, Pierce™ C18 Spin Columns, or Pierce™ C18 Tips).

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision	Date	Description
B.0	21 December 2022	The format and content were updated.
A.0	17 October 2015	New document for the Mass Spectrometry-Grade Endoproteinases.