

# Mass Spectrometry-Grade Endoproteinases

2072.3

Number	Description
90051	Lys-C Endoproteinase, MS Grade, 20 $\mu$ g
90053	Asp-N Endoproteinase, MS Grade, 2 $\mu$ g
90054	Glu-C Endoproteinase, MS Grade, 5 $\times$ 10 $\mu$ g
90055	Trypsin Endoproteinase, modified, TPCK treated, MS Grade, 5 $\times$ 20 $\mu$ g
90056	Chymotrypsin Endoproteinase, TLCK treated, MS Grade, 4 $\times$ 25 $\mu$ g

**Storage:** Upon receipt store at -20°C in a nonfrost-free freezer. Products are shipped with ice.

## Introduction

The Thermo Scientific Mass Spectrometry-Grade Endoproteinases offer specific cleavage at multiple sites (Table 1) that enable exploration of primary protein structure. 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.<sup>1</sup>

This improvement in sequence coverage and overall protein characterization can result from generating overlapping peptides, improving peptide chromatographic or ionization properties, or improving fragmentation efficiency. 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 new MS fragmentation techniques like electron transfer dissociation (ETD), leaving post-translational modifications intact.<sup>2</sup>

**Table 1. Cleavage Sites for the Thermo Scientific Mass Spectrometry-Grade Endoproteinases.**

Protease	Cleavage Specificity
Trypsin	Carboxyl side of arginine and lysine residues
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

## Important Product 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.
- Trypsin is a serine protease that specifically cleaves bonds at the carboxyl side of lysine and arginine residues; however, cleavage can be slowed or blocked in a sequence-dependent manner (i.e., near proline residue or with multiple adjacent cut sites). Peptide fragments with one missed cut are common and should be considered during mass analysis.
- The Modified Trypsin displays limited autolytic activity. The preparation is also treated with tosyl phenylalanyl chloromethyl ketone (TPCK) to eliminate residual contaminating chymotrypsin activity. TPCK does not interfere with mass spectral analysis. Using standard conditions, the most common trypsin fragment has a mass of 842.51 (m/z, M+H). This Modified Trypsin peptide can be used as an internal standard.

- Chymotrypsin is treated with N-alpha-p-tosyl-L-lysine chloromethyl ketone (TLCK) to eliminate residual trypsin interference. Chymotrypsin activity is improved by adding 10mM calcium chloride.<sup>3</sup>
- 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 8M 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 bicarbonate 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.<sup>14</sup>
- Asp-N is a metalloprotease and requires small quantities of zinc to enhance activity.<sup>5,6</sup>
- Although trypsin digestion is typically performed with aqueous solvents, using 80% acetonitrile is reported to improve enzyme activity and specificity in some circumstances.<sup>7</sup>

## Additional Materials Required

- Urea (Thermo Scientific, Product No. 29700)
- Ammonium bicarbonate (Acros, Product No. 370930250)
- DTT (Thermo Scientific Dithiothreitol, Product No. 20290 or 20291)
- Iodoacetamide (Acros, Product No. 122270050)
- Calcium chloride (Fisher, Product No. C79-500)
- Hydrochloric acid (Acros, Product No. 124635001)
- Zinc acetate (Acros, Product No. 370080250)
- Tris•HCl (Acros, Product No. 228030050)
- High purity/MS grade water (Fisher, Product No. W6-4)

## Material Preparation

Reducing Reagent	Dissolve 7.7mg of dithiothreitol (DTT) in 1mL of ultrapure water for a final concentration of 500mM. Transfer solution to a labeled microcentrifuge tube. Store aliquots of Reducing Reagent at -20°C. If using No-Weigh DTT (Product No. 20291), add 100µL of ultrapure water to one tube.
Digestion Buffer	Prepare the appropriate Digestion Buffer for the specific protease (see Table 2). Typically, 5X ammonium bicarbonate (500mM, pH 8.0) buffer is diluted with the protein sample and water to the final volume.
Enzyme Reconstitution	Prepare the appropriate Reconstitution Solution for the specific protease (see Table 2). Dissolve protease in a sufficient volume to yield a 1mg/ml final concentration. A typical final protease-to-protein substrate ratio is 1:20. Aliquot remaining reconstituted enzyme in single-use volumes and store at -80°C.

**Table 2. Enzyme-specific reconstitution solutions and digestion buffers.**

<b>Enzyme</b>	<b>Enzyme Reconstitution Solution</b>	<b>5X Digestion Buffer</b>
Trypsin	50mM acetic acid	500mM ammonium bicarbonate (pH 8.0)
Chymotrypsin	1mM HCl	500mM Tris•HCl (pH 8.0), 10mM calcium chloride
Lys-C	Ultrapure water	500mM ammonium bicarbonate (pH 8.0) or 2M urea
Glu-C	Ultrapure water	500mM ammonium bicarbonate (pH 8.0) or 500mM ammonium acetate (pH 4.0)
Asp-N	Ultrapure water	250mM Tris•HCl with 2.5mM zinc acetate (pH 8.0)

## Procedure for Protein Digestion

### A. Reduction and Alkylation

- Using a 0.5-0.7mL microcentrifuge tube, add 5 $\mu$ L of protein (1-50 $\mu$ g), 25 $\mu$ L of ultrapure water and 10 $\mu$ L of 5X Digestion Buffer.
- Adjust solution pH to ~8.0 (or to pH 4.0 for Glu-C).
- Add 2 $\mu$ L of 500mM DTT for a final concentration of 10mM and mix. Incubate at 60°C for 45 minutes.
- Cool to room temperature.
- Immediately before use, add 92mg of iodoacetamide (IAM) to a foil-wrapped tube. Add 1mL of ultrapure water to the tube for a final concentration of 500mM IAM.
- Add 3.5 $\mu$ L of 500mM IAM to the reduced protein sample for a final concentration of 20mM. Vortex and centrifuge briefly. Discard any remaining reconstituted IAM.
- Incubate the reaction at room temperature for 30 minutes protected from light.
- Add 1 $\mu$ L of 500mM DTT to quench the alkylation reaction.

### B. Digestion

- Adjust the volume of each sample to 50 $\mu$ L with ultrapure water.
- Add 2.5 $\mu$ L of 1mg/ml protease for a final 1:20 enzyme-to-protein sample ratio. Mix the reaction.
- Incubate the tube at 37°C for 16-24 hours (overnight).
- Centrifuge the tube briefly to collect all liquid in the base of the tube.
- Store samples at -80°C until mass spectrometric analysis. Immediately before analysis, cleanup samples with C18 spin columns (Thermo Scientific C18 Spin Tips, Product No 84850).

## Troubleshooting

Problem	Possible Cause	Solution
No digestion	Incorrect pH or buffer conditions	Use the specific buffer as listed in Table 2
	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 10mM 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 Bioworks or Proteome Discoverer Software)
Over-alkylation	Alkylation was allowed to proceed for too long	Alkylate at room temperature for 30 minutes and quench reaction with 10mM 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	Cleanup sample before analysis with reversed-phase tips or spin cartridges (i.e., PepClean C18 Spin Columns)

## Related Thermo Scientific Products

84840	Mass Spec Sample Prep Kit for Cultured Cells, 20-rxn kit
20233	Immobilized TPCK Trypsin, 50mg
89895	In-Solution Tryptic Digestion and Guanidination Kit
89871	In-Gel Tryptic Digestion Kit, sufficient reagents for approximately 150 in-gel digestions
84850	C18 Spin Tips, 96/pkg
28904	Trifluoroacetic Acid, Sequanal Grade, 10 × 1mL
28905	Formic Acid, 10 × 1mL
89853	Phosphopeptide Isolation Kit
90008	Pierce Strong Cation Ion Exchange Columns, mini
90010	Pierce Strong Anion Ion Exchange Columns, mini

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## General References

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