INSTRUCTIONS

Pierce LysN Protease, MS Grade

90300 90301

2570.1

Number	Description
90300	**Pierce LysN Protease, MS Grade,** 20µg
90301	**Pierce LysN Protease, MS Grade,** 5 × 20µg

Storage: Upon receipt, store at -20°C. Product is shipped on ice packs.

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Introduction

Thermo Scientific™ Pierce™ LysN Protease is a mass spectrometry (MS)-grade metalloprotease that cleaves at the amino-side of lysine residues. LysN is a zinc metalloprotease derived from *Grifola frondosa* that is resistant to moderate denaturing conditions making it ideal for digestion of samples for "shotgun" proteomics. Peptides generated by LysN are longer than those generated by trypsin and have more prevalent charged amino-terminal peptide fragments which are useful for *de novo* sequencing with collision induced dissociation (CID). The purified native Pierce LysN Protease has improved stability, specific activity and cleavage selectivity.

Important Product Information

- Maximal LysN activity occurs at pH 7-9. Common digestion buffers include 50mM ammonium bicarbonate, pH 8; 50mM Tris, pH 8; and 50mM TEAB, pH 8.5.
- LysN remains active under moderate denaturing conditions including 0.1% SDS, 6M urea or heat up to 70°C.
- EDTA or other metal chelators interfere with LysN activity. To recover activity loss due to the presence of metal chelators, add a zinc salt such as $ZnSO_4$ at a concentration greater than the metal chelator to the digestion buffer.
- Reconstituted stock solutions of LysN in water are stable at -80°C for 2 years or -20°C for one year without significant loss in activity. Minimize the number of freeze/thaw cycles by aliquoting stock solutions of the enzyme.
- Reduction and alkylation of cysteine residues using dithiothreitol (DTT) and iodoacetamide (IAA), respectively, will cleave disulfide bonds and prevent disulfide bond reformation. These steps improve digestion of cysteine-containing proteins and detection of cysteine-containing peptides. Alkylation with IAA increases the mass of a peptide by 57.02Da for each cysteine present.

Material Preparation

Enzyme Reconstitution	Reconstitute Pierce LysN Protease using LC/MS grade water to 0.5mg/mL (i.e., add 40µL LC/MS grade water to 20µg of lyophilized LysN). Aliquot reconstituted enzyme in single-use volumes and store at -20°C or -80°C.

Procedure for In-solution Protein Digestion

Note: The following protocol is an example application for this product. Specific applications will require optimization.

A. Additional Materials Required

- 1M Tris, pH 8 (e.g., Fisher Scientific Product No. BP1758-100)
- Urea, sequanal grade (e.g., Thermo Scientific Product No. 29700)
- Ammonium bicarbonate (e.g., Acros Product No. 370930250)
- DTT (e.g., Thermo Scientific Product No. 20290 or 20291)
- IAA (e.g., Thermo Scientific Product No. 90034)
- LC/MS grade water (e.g., Thermo Scientific Product No. 51140)
- Optional: 0.5M TCEP (e.g., Thermo Scientific Product No. 77720)
- Optional: SDS (e.g., Fisher Scientific Product No. BP1311-1)
- Optional: Thermo Scientific™ Pierce™ C18 Spin Columns (Product No. 89870)

B. Reduction and Alkylation

1. Dissolve protein in 50mM ammonium bicarbonate or 50mM Tris, pH 7.5-8 with a denaturing reagent such as 8M urea or 0.1% SDS.

   Note: Sodium deoxycholate is not recommended as a denaturing reagent because it reduces LysN activity.
2. Prepare a fresh solution of 500mM DTT by dissolving 7.7mg of DTT in 100µL of ultrapure water.
3. Add 500mM DTT solution to the protein sample to a final concentration of 20mM (1:25 dilution) and mix briefly.
4. Incubate at 37°C for 45 minutes or 95°C for 10 minutes.
5. Prepare a fresh solution of 1M IAA by dissolving 93mg of IAA in 500µL of ultrapure water.

   Note: Protect IAA stock solutions from light.
6. Add 1M IAA solution to the reduced protein sample to a final concentration of 40mM (1:25 dilution) and mix briefly.
7. Incubate the reaction mixture at room temperature for 15 minutes protected from light.
8. Quench the alkylation reaction by adding 500mM DTT solution to a final concentration of 10mM (1:50 dilution).

C. Digestion

1. Add reconstituted LysN solution to the sample to a final protease-to-protein ratio of 1:75 to 1:100 (w/w).

   Note: Protein samples dissolved in 8M urea must be diluted to ≤ 6M urea before digestion. For SDS-containing samples, dilution is not necessary.
2. Incubate the sample at 50°C for 2 hours or 37°C for 4 hours.

   Note: Digestion can also be performed at 70°C for 45 minutes; however, more missed cleavages will occur.
3. Store samples at -20°C to stop digestion reactions. Immediately before MS analysis, clean-up samples with C18 spin columns (Pierce C18 Spin Columns, Product No 89870) ensuring to acidify the sample with formic acid for proper C18 binding.

Troubleshooting

Problem	Possible Cause	Solution
No or incomplete digestion	Incorrect pH or buffer conditions	Check buffer pH
	Reduced enzymatic activity	Once reconstituted, store enzyme at -80°C for 1 year. Do not let enzyme sit at room temperature
		Ensure no metal chelator is present or add a Zn^{2+} salt
	Too little enzyme used	Increase amount of LysN for digestion
	Too high incubation temperature used	Decrease incubation temperature
Low sequence coverage	Too few, too many or unevenly distributed protease digestion sites	Separately use multiple proteases to digest the sample and combine results (e.g., multi-consensus reports in Thermo Scientific™ Proteome Discoverer Software)
Precipitation after alkylation	Too much reduction/alkylation buffer for quantity of protein being digested	Quench alkylation reaction using 10mM DTT
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 during LC-MS	Buffer, salt or urea interference	Clean-up sample before analysis with reversed-phase tips or spin cartridges (e.g., Pierce C18 Spin Columns)

Related Thermo Scientific Products

90057 **Pierce Trypsin Protease, MS Grade,** 5 × 20µg/vial

90058 **Pierce Trypsin Protease, MS Grade,** 5 × 100µg/vial

90059 **Pierce Trypsin Protease, MS Grade,** 1mg/vial

84849 **Pierce Mass Spec Sample Prep Kit for Cultured Cells**

20233 **Immobilized TPCK Trypsin,** 50mg

90051 **Lys-C Endoproteinase, MS Grade,** 20µg

90054 **Glu-C Endoproteinase, MS Grade,** 5 × 10µg

90053 **Asp-N Endoproteinase, MS Grade,** 2µg

90056 **Chymotrypsin Endoproteinase, TLCK treated, MS Grade,** 4 × 25µg

89895 **In-Solution Tryptic Digestion and Guanidination Kit**

89870 **Pierce C18 Spin Columns,** 25/pkg

28904 **Trifluoroacetic Acid, Sequanal Grade,** 10 × 1mL

28905	**Formic Acid,** 10 × 1mL
88300	**Pierce Fe-NTA Phosphopeptide Enrichment Kit**
88301	**Pierce TiO_2 Phosphopeptide Enrichment and Clean-up Kit**
88302	**Pierce Graphite Spin Columns,** 30 columns

General References

Taouatas, N., *et al.* (2010). Evaluation of metalloendopeptidase Lys-N protease performance under different sample handling conditions. *J Proteome Res.* **9(8):**4282-8.

Gershon, P. (2014). Cleaved and missed sites for trypsin, Lys-C, and Lys-N can be predicted with high confidence on the basis of sequence content. *J Proteome Res.* **13(2):**702-9.

Nonaka, T. J., *et al*. (1998). Kinetic characterization of lysine-specific metalloendopeptidases from *Grifola frondosa* and *Pleurotus ostreatus* fruiting bodies. *Biochemistry* **124(1):**157-62.