INSTRUCTIONS

Pierce Trypsin Protease, MS Grade

90057 90058 90059 90305

2456.1

Number	Description
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
90305	**Pierce Trypsin Protease, MS Grade,** (1mg/1mL, frozen liquid)

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

Introduction

Effective protein characterization and identification by mass spectrometry (MS) begins with protein digestion. Trypsin is the protease of choice for accomplishing this task. Thermo Scientific™ Pierce™ Trypsin Protease is a mass spectrometry (MS)-grade serine endoproteinase that cleaves at the carboxyl-end of lysine and arginine residues with very high selectivity. Pierce Trypsin Protease has been purified and chemically modified to improve its stability, specific activity and cleavage selectivity.

Important Product Information

- Pierce Trypsin Protease is treated with TPCK to eliminate chymotrypsin activity and methylated at lysine residues to reduce auto-catalytic activity.
- Maximal trypsin activity occurs at pH 7-9; the enzyme is reversibly inactivated at pH < 4. Common digestion buffers include 50mM ammonium bicarbonate, pH 8; 50mM Tris, pH 8; and 50mM TEAB, pH 8.5.
- Trypsin is resistant to mild denaturing conditions including 0.1% SDS, 1M urea or 10% acetonitrile (ACN), which may be used to facilitate digestion.
- High monovalent salt concentrations (i.e., > 100mM NaCl) may interfere with trypsin activity. Addition of 1mM $CaCl_2$ to digestion buffers is optional and may improve the activity of modified trypsin.
- Reconstituted stock solutions of trypsin in 50mM acetic acid are stable at -20°C for > 1 year without significant loss in activity. Minimize the number of stock solution freeze/thaw cycles by aliquoting stock solutions of enzyme. Store reconstituted trypsin stock solutions at -80°C in single-use volumes for longer-term stability.
- Reduction and alkylation of cysteine residues using dithiothreitol (DTT) and iodoacetamide (IAA), respectively, will cleave disulfide bonds and prevent disulfide bond reformation. This improves 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 Preparation	Reconstitute lyophilized trypsin using 50mM acetic acid to 1mg/mL (i.e., add 20µL of 50mM acetic acid to 20µg of lyophilized trypsin). Aliquot reconstituted enzyme in single-use volumes and store at -80°C. Frozen liquid trypsin can be used as provided or aliquoted in single-use volumes and stored at -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)
- Acetic acid (e.g., Fisher Scientific Product No. A35-500)
- 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, pH 8 or a denaturing buffer such as 50mM Tris, pH 8 containing 8M urea or 0.1% SDS.

   Note: Use denaturing buffers for full protein reduction, alkylation and digestion.
2. Prepare a new solution of 500mM DTT by dissolving 7.7mg of DTT in 100µL of ultrapure water.
3. Add 500mM DTT solution to protein sample to a final concentration of 20mM (1:25 dilution) and mix briefly.
4. Incubate at 60°C for 1 hour 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 30 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 trypsin solution to the sample to a final protease to protein ratio of 1:20 to 1:100 (w/w).

   Note: Protein samples dissolved in 8M urea must be diluted to < 1M urea before digestion. For SDS-containing samples, dilution is not necessary.
2. Incubate the tube at 37°C for 4-24 hours.
3. Store samples at -20°C to stop digestion reactions. Immediately before MS analysis, clean-up samples with C18 spin columns (e.g., Pierce C18 Spin Columns, Product No 89870).

Procedure for In-Gel Protein Digestion

Note: This procedure is for colloidal coomassie-stained or fluorescent dye-stained acrylamide gel slices. Alternative destaining procedures are required for silver- or zinc-stained protein bands. Use sufficient reagent volumes to completely cover gel-slice pieces for all steps. Use LC/MS-grade reagents, clean containers and gloves to minimize contamination.

A. Additional Materials Required

- Ammonium bicarbonate (e.g., Acros Product No. 370930250)
- 0.5M TCEP (e.g., Thermo Scientific Product No. 77720)
- IAA (e.g., Thermo Scientific Product No. 90034)
- Acetic acid (e.g., Fisher Scientific Product No. A35-500)
- LC/MS-grade water (e.g., Thermo Scientific Product No. 51140)
- Trifluoroacetic acid (TFA), sequencing grade (e.g., Thermo Scientific Product No. 28904)
- Acetonitrile (ACN) (e.g., Thermo Scientific Product No. 51101)
- Pierce C18 Spin Columns (Product No. 89870)
- Vacuum concentrator (e.g., Thermo Scientific™ SpeedVac™ Vacuum Concentrator)

B. SDS-PAGE and Destaining

1. Separate proteins by SDS-PAGE and stain gel using a reversible, colloidal coomassie stain such as Thermo Scientific™ GelCode™ Blue Stain (Product No. 24590).
2. Using a clean razor blade, cut gel slices containing stained proteins and transfer 1 × 1mm pieces of gel to a microcentrifuge tube.
3. Add 200µL of 100mM ammonium bicarbonate/50% ACN to gel slices and incubate at 37°C for 30 minutes to destain the gel slices.
4. Remove destaining buffer and repeat Step 3 twice or until all stain is removed.

C. Reduction and Alkylation (Optional)

1. Prepare new 5mM TCEP solution by diluting 10µL of 0.5M TCEP in 1mL of 100mM ammonium bicarbonate.
2. Add 5mM TCEP solution to the destained gel slices and incubate at 60°C for 10 minutes.
3. Prepare new 100mM IAA solution by dissolving 9.3mg iodoacetamide in 1mL of 100mM ammonium bicarbonate.
4. Remove TCEP solution from the gel slices. Add 100mM IAA solution and incubate sample at 37°C for 15 minutes with shaking.
5. Remove IAA solution from gel slices. Rinse gel slices with 100mM ammonium bicarbonate/50% ACN and incubate sample at 37°C for 15 minutes with shaking.
6. Repeat Step 5 twice to remove excess IAA from gel slices.

D. Digestion

1. Shrink gel pieces by adding 50µL of ACN. Incubate sample for 15 minutes at room temperature.
2. Remove ACN and allow gel pieces to air dry for 5-10 minutes.
3. Dilute 1mg/mL trypsin stock solution to 0.01mg/mL using 100mM ammonium bicarbonate (1:100 dilution).
4. Add 50µL of 0.01mg/mL trypsin solution to the sample and incubate the tube at 37°C for 8-24 hours.
5. Remove the digest solution and transfer to a new microcentrifuge tube.
6. Extract the gel pieces three times by adding 50µL of 50% ACN/0.1% TFA solution and incubating at 37°C for 5-15 minutes.
7. Combine gel extracts with digest and evaporate the liquid using a vacuum concentrator.
8. Clean-up samples with C18 spin columns (e.g., Pierce C18 Spin Columns, Product No 89870).

Troubleshooting

Problem	Possible Cause	Solution
No digestion	Incorrect pH or buffer conditions	Check buffer pH
	Reduced enzymatic activity	Reconstitute enzyme immediately before use and make single-use volumes 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
		Digest the sample with Lys-C protease before digestion with trypsin
	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)
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	Buffers, 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

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

90300 **LysN Protease, MS Grade,** 20µg

90301 **LysN Protease, MS Grade,** 5 x 20µg

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

89871 **In-Gel Tryptic Digestion Kit**

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

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

28905 **Formic Acid,** 10 × 1mL

General References

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Galvani, M., *et al*. (2001). Alkylation kinetics of proteins in preparation for two-dimensional maps: A matrix assisted desorption/ionization-time of flight-mass spectrometry investigation. *Electrophoresis* **22:**2058-65.

Herbert, B., *et al*. (2001). Reduction and alkylation of proteins in preparation of two-dimensional map analysis: Why, when, and how? *Electrophoresis* **22:**2046-57.

Sechl, S. and Chait, B.T. (1998). Modification of cysteine residues by alkylation. A tool in peptide mapping and protein identification. *Anal Chem* **70:**5150-8.

Shevchenko, A., *et al*. (1996). Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. *Anal Chem* **68:**850-8.

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Speicher, K.D., *et al*. (2000). Systematic analysis of peptide recoveries from in-gel digestions for protein identifications in proteome studies. *J Biomol Tech* **11:**74-86.

Tarentino, A.L., *et al.* (1995). Molecular cloning and sequence analysis of flavastacin: An O-glycosylated prokaryotic zinc metalloendopeptidase. *Arch Biochem Biophys* **319:**281-5.