thermo scientific USER GUIDE

Pierce™ Trypsin/Lys-C Protease Mix, MS Grade

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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

Protein characterization, identification and quantification by mass spectrometry (MS) begins with efficient, reproducible protein digestion. Although trypsin is routinely used for protein digestion, this protease alone is not sufficient to fully digest proteins at the carboxyl-end of lysine and arginine residues. Therefore, Lys-C protease is commonly combined with trypsin to sequentially digest proteins with fewer missed cleavages. Thermo Scientific™ Pierce™ Trypsin/Lys-C Protease Mix, MS Grade is a mass spectrometry (MS)-grade serine endoproteinase mixture formulated for concurrent protein digestion providing more efficient digestion than with trypsin alone.

Contents

Product	Cat. No.	Contents	Storage
Pierce™ Trypsin/Lys-C Protease Mix, MS Grade	A40007	20 µg/vial	Store at -20°C in a frost-free freezer.
	A41007	5 × 20 µg/vial	
	A41009	100 µg/vial	

Additional information

- Reconstituted stock solutions of trypsin/Lys-C in 0.1% acetic acid are stable at 4°C for 1 month without significant loss in activity. Store reconstituted trypsin/Lys-C stock solutions at -20°C or -80°C in single-use volumes for longer-term stability. Minimize the number of stock solution freeze/thaw cycles by aliquoting stock solutions of enzyme mix.
- Maximal trypsin/Lys-C activity occurs at pH 7-9; the protease mix is reversibly inactivated at pH < 4. Common digestion buffers include ammonium bicarbonate, Tris, HEPES, and TEAB at 20-100 mM.
- Trypsin/Lys-C is resistant to mild denaturing conditions including 0.1% SDS, 1M urea or 10% acetonitrile (ACN), which may be used to facilitate digestion. Use of 6-8 M urea can reversibly inactivate trypsin for sequential Lys-C, tryptic digestion.
- High monovalent salt concentrations (i.e., >100mM NaCl) may interfere with protease mix activity. Addition of 1-10 mM $CaCl_2$ to digestion buffers is optional and may improve digestion efficiency for some proteins.
- Reduction with dithiothreitol (DTT) or tris(2-carboxyethyl)phosphine (TCEP) and alkylation with iodoacetamide (IAA) or chloroacetamide (CAA), respectively, will cleave disulfide bonds and modify cysteine residues to prevent disulfide bond reformation. This improves digestion of cysteine-containing proteins and detection of cysteine-containing peptides. Alkylation with IAA or CAA increases the mass of a peptide by 57.02 Da for each cysteine present.

Prepare enzyme mix

Reconstitute lyophilized trypsin/Lys-C in 0.1% acetic acid to 1 mg/mL (i.e., add 20 µL of 0.1% acetic acid to 20 µg of lyophilized trypsin/Lys-C mix). Aliquot reconstituted enzyme mix in single-use volumes and store at -80°C.

Perform In-solution protein digestion

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

Materials required but not supplied

- 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 Organics™ 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)

For Research Use Only. Not for use in diagnostic procedures.

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Reduce and alkylate

1. Dissolve protein in 50 mM ammonium bicarbonate, pH 8 or a denaturing buffer such as 50 mM Tris, pH 8 containing 8 M urea or 0.1% SDS.

   Note: Use denaturing buffers for full protein reduction, alkylation and digestion.
2. Prepare a fresh solution of 500 mM DTT by dissolving 7.7mg of DTT in 100µL of ultrapure water.
3. Add 500 mM DTT solution to protein sample to a final concentration of 20 mM (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 1 M IAA by dissolving 93 mg of IAA in 500 µL of ultrapure water.

   Note: Protect IAA stock solutions from light.
6. Add 1 M IAA solution to the reduced protein sample to a final concentration of 40 mM (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 500 mM DTT solution to a final concentration of 10 mM (1:50 dilution).

Clean-up protein

1. Use acetone precipitation to clean-up the protein sample prior to digestion by mixing the sample solution with 6 volumes of cold acetone and incubating at -20°C for 6-16 hours.
2. Centrifuge the suspension at 16,000 × *g* for 5 minutes to collect a protein pellet at the bottom of the tube.
3. Dissolve the reduced/alkylated protein pellet in your digestion buffer, such as 50 mM ammonium bicarbonate, pH 8 or a denaturing buffer such as 50 mM Tris, pH 8.

Digest

1. Add trypsin/Lys-C solution to the sample to a final protease to protein ratio of 1:20 to 1:100 (w/w).

   Note: Protein samples dissolved in 8 M urea must be diluted to <1 M urea for complete digestion. For SDS-containing samples (<0.1%), dilution is not necessary.
2. Incubate the sample at 37°C for 2-16 hours.
3. Store samples at -20°C or acidify by adding formic acid to pH <3 to stop digestion reactions. Before MS analysis, clean up samples with reversed-phase spin columns (e.g., Pierce™ Peptide Desalting Spin Columns, Product No. 89852).

Perform 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.

Materials required but not supplied

- Ammonium bicarbonate (e.g., ACROS Organics™ Product No. 370930250)
- 0.5 M 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., Fisher Scientific™ Product No. 51101)
- Pierce™ C18 Spin Columns (Product No. 89870)
- Vacuum concentrator (e.g., Thermo Scientific™ SpeedVac™ Vacuum Concentrator)

Perform SDS-PAGE and destain

1. Separate proteins by SDS-PAGE and stain gel using a reversible, colloidal coomassie stain such as Thermo Scientific™ GelCode™ Blue Stain Reagent (Product No. 24590).
2. Using a clean razor blade, cut gel slices containing stained proteins and transfer 1 × 1 mm pieces of gel to a microcentrifuge tube.
3. Add 200 µL of 100 mM 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 the previous step twice or until all stain is removed.

Reduce and alkylate *(Optional)*

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

Digest

1. Shrink gel pieces by adding 50 µL 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 1 mg/mL trypsin/Lys-C stock solution to 0.01 mg/mL using 100 mM ammonium bicarbonate (1:100 dilution).
4. Add 50 µL of 0.01 mg/mL trypsin/Lys-C solution to the sample and incubate the tube at 37°C for 4-24 hours.
5. Remove the digest solution and transfer to a new microcentrifuge tube.
6. Extract the gel pieces 3 times by adding 50 µL 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

Observation	Possible cause	Recommended action
Excess 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).
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.
Incomplete sequence coverage.	Incomplete digestion.	Reconstitute enzyme mix immediately before use and use the appropriate digestion buffer.
		Add mild denaturants, such as 0.1% SDS, 1 M urea or 10% ACN to facilitate digestion.
	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.
No digestion.	Incorrect pH or buffer conditions.	Verify buffer pH is between 7-9.
	Reduced enzymatic activity.	Reconstitute enzyme mix immediately before use and make single-use volumes to avoid multiple freeze/thaw cycles.
Over-alkylation.	Alkylation was allowed to proceed for too long.	Alkylate at room temperature for 30 minutes and quench reaction with 10 mM DTT.
Precipitation after alkylation.	Too much reduction/alkylation buffer for quantity of protein being digested.	Quench alkylation reaction using 10 mM DTT.

Related products

Product	Product No.
EasyPep™ Mini MS Sample Prep Kit	A40006
Pierce™ Trypsin Protease, MS Grade	90057
Lys-C Endoproteinase, MS Grade	90051
Glu-C Endoproteinase, MS Grade	90054
Asp-N Endoproteinase, MS Grade	90053
Chymotrypsin Endoproteinase, TLCK treated, MS Grade	90056
LysN Protease, MS Grade	90300
Pierce™ Trifluoroacetic Acid (TFA), Sequencing Grade	28904
Formic Acid, LC-MS Grade	28905

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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