Pierce™ Trypsin Protease, MS Grade

Catalog Numbers 90057, 90058, 90059, 90305

Doc. Part No. 2162456 Pub. No. MAN0011821 Rev. B.0

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

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, MS Grade 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, MS Grade has been purified and chemically modified to improve its stability, specific activity, and cleavage selectivity.

Contents and storage

Item	Cat. No.	Amount	Storage
Pierce™ Trypsin Protease, MS Grade	90057	5 x 20 µg	–20°C
	90058	5 x 100 µg	
	90059	1 mg	
	90305	100 µg at 1 mg/mL, frozen liquid	

Additional information

- Pierce™ Trypsin Protease, MS Grade 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 50 mM ammonium bicarbonate, pH 8; 50 mM Tris, pH 8; and 50 mM TEAB, pH 8.5.
- Trypsin is resistant to mild denaturing conditions including 0.1% SDS, 1 M urea, or 10% acetonitrile (ACN), which may be used to facilitate digestion.
- High monovalent salt concentrations (i.e., >100 mM NaCl) may interfere with trypsin activity. Addition of 1 mM $CaCl_2$ to digestion buffers is optional and may improve the activity of modified trypsin.
- Reconstituted stock solutions of trypsin in 50 mM 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 57.02 Da for each cysteine present.

Before you begin

Prepare enzyme

1. Reconstitute lyophilized trypsin using 50 mM acetic acid to 1 mg/mL (i.e., add 20 µL of 50 mM acetic acid to 20 µg of lyophilized trypsin).
2. 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.

Perform in-solution protein digestion

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

Required materials not supplied

- 1 M Tris, pH 8 (e.g., Cat. No. BP1758-100)
- Urea, sequanal grade (e.g., Cat. No. 29700)
- Ammonium bicarbonate (e.g., Cat. No. 370930250)
- DTT (e.g., Cat. No. 20290)
- IAA (e.g., Cat. No. A39271)
- Acetic acid (e.g., Cat. No. A35-500)
- LC/MS grade water (e.g., Cat. No. 51140)
- *(Optional)* 0.5 M TCEP (e.g., Cat. No. 77720)
- *(Optional)* SDS (e.g., Cat. No. BP1311-1)
- *(Optional)* Pierce™ C18 Spin Columns (Cat. No. 89870)

1 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 new solution of 500 mM DTT by dissolving 7.7 mg of DTT in 100 µL of ultrapure water.
3. Add 500 mM DTT solution to the 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. Alternatively, dissolve 9.3 mg of Pierce™ Iodoacetamide, Single-Use (Cat. No. A39271) in 50 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).

2 Digest protein in solution

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 8 M urea must be diluted to <1 M urea before digestion. For SDS-containing samples, dilution is not necessary.

2	Digest protein in solution *(continued)*	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, Cat. No. 89870).

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.

Required materials not supplied

- Ammonium bicarbonate (e.g., Cat. No. 370930250)
- 0.5 M TCEP (e.g., Cat. No. 77720)
- IAA (e.g., Cat. No. A39271)
- Acetic acid (e.g., Cat. No. A35-500)
- LC/MS-grade water (e.g., Cat. No. 51140)
- Trifluoroacetic acid (TFA), sequencing grade (e.g., Cat. No. 28904)
- Acetonitrile (ACN) (e.g., Cat. No. 51101)
- Pierce™ C18 Spin Columns (Cat. No. 89870)
- Vacuum concentrator (e.g., SpeedVac™ Vacuum Concentrator)

1	**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 (Cat. No. 24590). 2. Using a clean razor blade, cut gel slices containing stained proteins and transfer 1 mm x 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 step 1.3 twice or until all stain is removed.
2	***(Optional)* Reduce and alkylate**	1. Prepare a 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 a new 100 mM IAA solution by dissolving 9.3 mg iodoacetamide in 500 µL of 100 mM ammonium bicarbonate. 4. Remove TCEP solution from the gel slices. Add 100 mM IAA solution and incubate the sample at 37°C for 15 minutes with shaking. 5. Remove IAA solution from the gel slices. Rinse gel slices with 100 mM ammonium bicarbonate/50% ACN and incubate the sample at 37°C for 15 minutes with shaking. 6. Repeat step 2.5 twice to remove excess IAA from gel slices.

3 Digest protein in-gel

1. Shrink gel pieces by adding 50 µL of ACN. Incubate the 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 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 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 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, Cat. No. 89870).

Troubleshooting

Observation	Possible cause	Recommended action
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 10 mM 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 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 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).

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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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	15 November 2022	The format and content were updated.
A.0	17 October 2015	New document for Pierce™ Trypsin Protease, MS Grade.