

EasyPep™ Maxi MS Sample Prep Kit

Catalog Numbers A45734

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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

The Thermo Scientific™ EasyPep™ Maxi MS Sample Prep Kit enables efficient and reproducible processing of cultured mammalian cells, plasma, and tissues for proteomic mass spectrometry (MS) analysis. The kit contains pre-formulated buffers, MS-grade enzyme mix, peptide clean-up columns, and an optimized protocol to generate MS-compatible peptide samples in less than 3 hours. The kit is optimized to process eight 0.5-2 mg protein samples resulting in high yield of MS-ready peptides. Alternatively, each Peptide Clean-Up Maxi Column can be used to process a combined, multiplex set of isobaric tag-labeled samples (10-100 µg each, ≤ 2 mg total). Some key features of the kit that reduce total sample preparation time include: addition of Universal Nuclease to reduce viscosity from nucleic acids without the need for sonication, a rapid "one pot" reduction/alkylation solution for cysteine modification (carbamidomethylation, +57.02), and a trypsin/Lys-C protease mix for more complete digestion. In addition, the kit includes peptide clean-up columns and buffers to prepare detergent-free peptide samples for direct LC-MS analysis or further sample processing such as isobaric tag (e.g., TMT™ or TMTpro reagent) labeling, phosphopeptide enrichment, or high pH reversed-phase fractionation.

Contents

Product	Cat. No.	Contents	Storage
EasyPep™ Maxi MS Sample Prep Kit	A45734	Kit sufficient for 8 preparations of 0.5-2 mg Contents: Lysis Solution, 25 mL Universal Nuclease, 25 kU Reduction Solution, 7 mL Alkylation Solution, 7 mL Enzyme Reconstitution Solution, 7 mL Pierce™ Trypsin/Lys-C Protease Mix, MS Grade, 8 × 100 µg Digestion Stop Solution, 7 mL Peptide Clean-Up Columns, 8 each Wash Solution A, 40 mL Wash Solution B, 3 × 27 mL Elution Solution, 2 × 20 mL Low Protein Binding Collection Tubes, 2 mL, 40 each	Store at 4°C. Enzyme components can be stored at -20°C.

Additional information

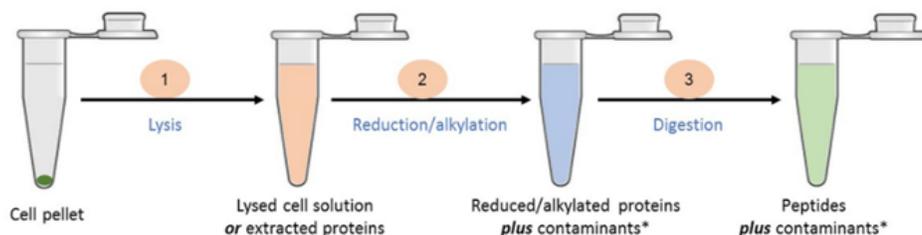
- Warm the Lysis Solution to room temperature before use. Store buffers and columns at 4°C.
- Addition of phosphatase inhibitors to Lysis Solution (e.g., Halt™ Phosphatase Inhibitor Cocktail, Product No. 78420) is recommended before cell lysis for phosphopeptide enrichment and analysis.
- Protease inhibitor cocktails without EDTA are recommended as the EDTA inhibits Universal Nuclease and Trypsin/Lys-C Protease Mix activity.
- For long term storage (>3 months), store Universal Nuclease and Trypsin/Lys-C Protease Mix at -20°C.
- After addition of Enzyme Reconstitution Solution, the Trypsin/Lys-C Protease Mix can be stored at 4°C for up to 1 month or -20°C for 1 year.
- Use of peptide clean-up columns is required to remove contaminants and enzymes before LC-MS analysis.

Materials required but not supplied

- (Optional) Tissue homogenizer
- 15 mL conical tubes
- Heat block or thermo mixer
- Protein assay kit (e.g., Thermo Scientific™ Pierce™ BCA Protein Assay Kit, Product No. 23227)
- Mass spectrometer with nano-flow liquid chromatography (LC) system
- (Optional) 20% formic acid (FA) and 5% hydroxylamine for TMT™ reagent labeling

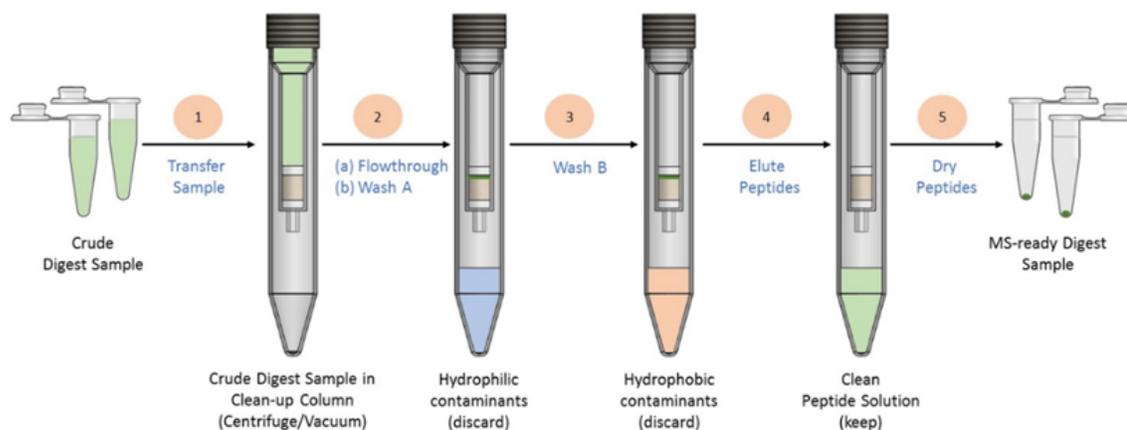
Procedure summary

Stage 1: Chemical & Enzymatic Sample Processing



*contaminants – hydrophilic/hydrophobic buffer salts, reagents, detergent, biomolecules other than proteins

Stage 2: Peptide Clean-up



Procedure for large-scale sample prep (unlabeled)

Note: Use 0.5-2 mg of protein per sample preparation. Rinse cultured cells or tissues 2-3 times with 1X PBS to remove cell culture media or excess blood, respectively. Resuspend proteins, cells or tissues in Lysis Solution without additional buffers.

Extract protein, reduce, and alkylate

- For cultured cells, add 1 mL of Lysis Buffer and 5 μL of Universal Nuclease to a minimum of 5×10^6 cells. Pipet up and down (with P1000 tip) for 10-15 cycles until sample viscosity is reduced.
Note: Centrifugation of cultured cell lysates is typically not required after aspiration using pipet.
- For tissue samples, add 1 mL of Lysis Solution (containing 5 μL Universal Nuclease) per 50 mg of tissue and disrupt with tissue homogenizer until sample is homogenized. Centrifuge tissue lysates at $16,000 \times g$ for 10 minutes.
- For purified proteins, serum, and plasma samples, dilute samples directly in 1 mL of Lysis Solution to 0.5-2 mg/mL. Use 5-30 μL of undepleted plasma or serum per sample preparation.
Note: For purified proteins and plasma samples, addition of Universal Nuclease is **not** required.
- Determine the protein concentration of the supernatant using established methods such as the Pierce™ BCA Protein Assay Kit (Product No. 23227) or Pierce™ Rapid Gold BCA Protein Assay Kit (Product No. A53226).
- Transfer 0.5-2 mg of protein sample into a new 2 mL microcentrifuge tube and adjust final volume to 1 mL with Lysis Solution.
- Add 500 μL of Reduction Solution to the sample and gently mix.
- Add 500 μL of Alkylation Solution to the sample and gently mix.
- Split samples into two 2-mL microcentrifuge tubes for a total of 1 mL each tube.
- Incubate samples at 95°C using a heat block for 10 minutes or 50° using a heat block for 30 minutes to reduce and alkylate the protein sample.
- After incubation, remove sample from the heat block to cool to room temperature.

Digest protein

- Add 100 μL of Enzyme Reconstitution Solution to 1 vial of Trypsin/Lys-C Protease Mix.
- Add 50 μL of the reconstituted enzyme solution to each tube of reduced and alkylated protein samples.
Note: Store unused reconstituted enzyme at 4°C for 1 month or -20°C for 1 year.
- Incubate with shaking at 37°C for 1-3 hours to digest the protein samples. We recommend 3 hours for plasma samples.
- After incubation is completed, add 250 μL of Digestion Stop Solution to acidify each of the samples and gently mix.

Clean-up peptides

Note: The Peptide Clean-Up Columns are compatible with centrifuge and vacuum manifold equipment. The protocol below describes the clean-up using a swinging-bucket centrifuge.

1. Remove the tab at the bottom of the Peptide Clean-Up Column, remove the cap, and place into a 15 mL conical tube.
2. Transfer the protein digest sample from both tubes (~3 mL total volume) into a dry Peptide Clean-Up Column.
3. Centrifuge at $2,000 \times g$ for 2 minutes. Discard the flowthrough.
4. Add 3 mL of the Wash Solution A into the column.
5. Centrifuge at $2,000 \times g$ for 2 minutes. Discard the flowthrough.
6. Add 3 mL of Wash Solution B into the column.
7. Centrifuge at $2,000 \times g$ for 2 minutes. Discard the flowthrough.
8. Repeat steps 6 and 7 for a total of 2 or 3 washes with Wash Solution B.
9. Transfer the Peptide Clean-Up Column into a new 15 mL conical tube.
10. Add 3 mL of the Elution Solution into the column.
11. Centrifuge at $2,000 \times g$ for 2 minutes to collect the clean peptide sample.
12. Transfer the eluted peptides to two 2-mL low protein binding tubes.
13. Dry the peptide sample using a vacuum centrifuge.
14. Resuspend the sample in 250-500 μL of 0.1% formic acid in water depending on starting amount for LC-MS analysis.
15. (Optional) Assess peptide yield and concentration using a quantitative peptide assay. Adjust the peptide concentration with 0.1% formic acid in water solution for optimal LC-MS column loading.

Procedure for multiplex sample prep (TMT[™]/TMTpro-labeled) using Maxi Peptide Clean-Up Column

Note: This procedure is for preparing and labeling 25-100 μg of individual samples to be combined into a single multiplex sample of 0.25-1.6 mg for clean up. Rinse cultured cells or tissues 2-3 times with 1X PBS to remove cell culture media or excess blood, respectively. Resuspend proteins, cells or tissues in Lysis Solution without additional buffers.

Extract protein, reduce, and alkylate

1. For cultured cells, add 100 μL of Lysis Buffer and 0.5 μL of Universal Nuclease to a minimum of 1×10^6 cells. Pipet up and down (with P200 tip) for 10-15 cycles until sample viscosity is reduced.
Note: Centrifugation of cultured cell lysates is typically not required after aspiration using pipet.
2. For tissue samples, add 100 μL of Lysis Solution (containing 0.5 μL Universal Nuclease) per 5 mg of tissue and disrupt with tissue homogenizer until sample is homogenized. Centrifuge tissue lysates at $16,000 \times g$ for 10 minutes.
3. For purified proteins, serum, and plasma samples, dilute samples directly in Lysis Solution to 25-100 $\mu\text{g}/\mu\text{L}$. Use 0.5-1.5 μL of undepleted plasma or serum per sample preparation.
Note: For purified proteins and plasma samples, addition of Universal Nuclease is **not** required.
4. Determine the protein concentration of the supernatant using established methods such as the Pierce[™] BCA Protein Assay Kit (Product No. 23227) or Pierce[™] Rapid Gold BCA Protein Assay Kit (Product No. A53226).
5. Transfer 25-100 μg of protein sample into a new 2 mL microcentrifuge tube and adjust final volume to 100 μL with Lysis Solution.
6. Add 50 μL of Reduction Solution to the sample and gently mix.
7. Add 50 μL of Alkylation Solution to the sample and gently mix.
8. Incubate sample at 95°C using heat block for 10 minutes or 50°C using heat block for 30 minutes to reduce and alkylate the protein sample. We recommend 95°C for plasma samples.
9. After incubation, remove sample from the heat block to cool to room temperature.

Digest protein

1. Add 500 μL of Enzyme Reconstitution Solution to 1 vial of Trypsin/Lys-C Protease Mix.
2. Add 25-50 μL of the reconstituted enzyme solution to both reduced and alkylated protein sample solutions.
Note: Store unused reconstituted enzyme at 4°C for 1 month or -20°C for 1 year.
3. Incubate with shaking at 37°C for 1-3 hours to digest the protein sample. We recommend 3 hours for plasma samples.
Note: Do not add Digestion Stop Solution.

Label protein digest with TMT[™] reagent

The protocol below describes labeling before peptide clean up using 1:4 to 1:8, w:w sample to TMT[™] reagent. For TMTpro reagents, use 1:5 to 1:10, w:w sample to tag.

1. For TMT[™]-labeled reagent, add 0.2-0.8 mg (for TMTpro reagent, add 0.25-1 mg) dissolved in 40 μL of 100% acetonitrile to each buffered peptide sample and incubate for 30-60 minutes at room temperature.
2. Add 50 μL of 5% hydroxylamine, 20% formic acid solution to each labeling reaction to quench and acidify. Verify pH < 4 using pH paper.
Note: The quench solution replaces the Digestion Stop Solution used in the label-free sample preparation workflow. No incubation is required.
3. Combine equal amounts of labeled peptide.

Clean up peptides

Note: The Peptide Clean-Up columns are compatible with centrifuge and vacuum manifold equipment. The protocol below describes the clean-up using a swinging-bucket centrifuge.

1. Remove the tab at the bottom of the Peptide Clean-up Column, remove the cap, and place into a 15 mL conical tube.
2. Transfer the protein digest sample (~3-4 mL total volume). If the volume exceeds 3 mL, load the remaining sample after the first centrifugation into the dry Peptide Clean-Up Column.
3. Centrifuge at $2,000 \times g$ for 2 minutes. Discard the flowthrough.
4. Add 3 mL of the Wash Solution A into the column.
5. Centrifuge at $2,000 \times g$ for 2 minutes. Discard the flowthrough.
6. Add 3 mL of the Wash Solution B into the column.
7. Centrifuge at $2,000 \times g$ for 2 minutes. Discard the flowthrough.
8. Repeat steps 6 and 7 for a total of 2 or 3 washes with Wash Solution B.
9. Transfer the Peptide Clean-Up Column into a new 15 mL conical tube.
10. Add 3 mL of the Elution Solution into the column.
11. Centrifuge at $2,000 \times g$ for 2 minutes to collect the clean peptide sample.
12. Transfer the eluted peptides to 2 mL low protein binding tubes.
13. Dry the peptide sample using a vacuum centrifuge.
14. Resuspend the sample in 250-500 μ L of 0.1% formic acid in water depending on the starting amount for LC-MS analysis.
15. (Optional) Assess peptide yield and concentration using a quantitative peptide assay. Adjust the peptide concentration with 0.1% formic acid in water solution for optimal LC-MS column loading.

Troubleshooting

Observation	Possible cause	Recommended action
High viscosity sample after lysis.	Universal Nuclease was not added.	Add 1 μ L of Universal Nuclease per 100 μ L of lysis buffer.
	Protease inhibitor cocktail with EDTA used.	Do not add protease inhibitor cocktails containing EDTA.
Incomplete digestion.	Inactive enzyme.	Store enzymes at 4°C for 1 month or -20°C for long-term stability.
		Cool samples after reduction/alkylation to room temperature before addition of protease mix.
	Insufficient digestion time.	Increase digestion time to 3 hours with shaking.
	Protease inhibitor cocktail used.	Do not add protease inhibitor cocktails.
Low protein yield.	Insufficient cells.	Increase the number of cells used for lysis.
Over-alkylation	Alkylation occurred for too long.	Alkylate at 90°C for 10 minutes or 50°C for 30 minutes.
Overestimation of peptide yield using peptide assays.	Incomplete removal of elution buffer during speedvac.	Speedvac eluted samples completely.
		Use Pierce™ Peptide Desalting Spin Columns (Product No. 89852) to remove excess buffer or TMT™ reagents.

Related products

Product	Product No.
EasyPep™ Mini MS Sample Prep Kit	A40006
EasyPep™ 96 MS Sample Prep Kit	A45733
EasyPep™ Lysis Buffer	A45735
Pierce™ BCA Protein Assay Kit	23225
Pierce™ Rapid Gold BCA Protein Assay Kit	A53225
Pierce™ Quantitative Colorimetric Peptide Assay Kit	23275
Pierce™ Quantitative Fluorometric Peptide Assay	23290
Pierce™ Trypsin/Lys-C Protease Mix, MS Grade	A40007

Limited product warranty

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