EasyPep™ 96 MS Sample Prep Kit

Catalog Numbers A45733

Doc. Part No. 2162739 **Pub. No.** MAN0018894 **Rev.** A.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

The Thermo Scientific™ EasyPep™ 96 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, a peptide clean-up plate, and an optimized protocol to generate MS-compatible peptide samples in less than 4 hours. The kit is optimized to process protein samples from 10-100 µg with high yield of MS-ready peptides. 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 a peptide clean-up plate and solutions to prepare detergent-free peptide samples for direct LC-MS analysis or further sample processing such as isobaric tag (e.g., TMT™ Reagent) labeling, phosphopeptide enrichment or high pH reversed-phase fractionation.

Contents

Product	Cat. No.	Contents	Storage
EasyPep™ 96 MS Sample Prep Kit	A45733	Kit sufficient for 96 preparations of 10-100 µg 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, 5 × 100 µg Digestion Stop Solution, 7 mL Collection Plate, 2 each Peptide Clean-Up Plate, 1 each Sample Prep Plate, 1 each Wash Solution A, 40 mL Wash Solution B, 3 × 27 mL Elution Solution, 2 × 20 mL	Store at 4°C. Enzyme components can be stored at -20°C.

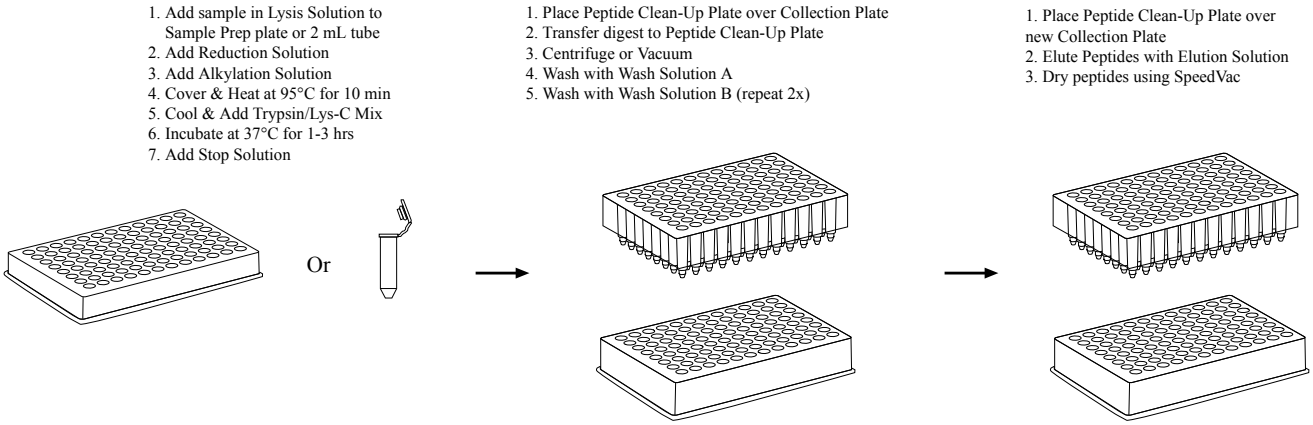
Additional information

- Warm the Lysis Solution to room temperature before use. Store solutions 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.
- Protease inhibitor cocktails without ETDA are recommended as the EDTA inhibits Universal Nuclease and Trypsin/LysC 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 Plate is required to remove contaminants and enzymes before LC-MS analysis. A partial plate (e.g., well, row, or column) can be used to clean up individual samples.
- Store Peptide Clean-Up Plate covered at 4°C or room temperature to prevent contamination.

Materials required but not supplied

- *(Optional)* Tissue homogenizer
- Sealing tape (e.g., Thermo Scientific™ Sealing Tape for 96-Well Plates, Product No. 15036)
- Heat block or thermo mixer
- Centrifuge with 96-well plate adaptor or vacuum manifold
- Protein assay kit (e.g., Thermo Scientific™ Pierce™ BCA Protein Assay Kit, Product No. 23227)
- *(Optional)* Peptide assay kit (e.g., Thermo Scientific™ Pierce™ Quantitative Colorimetric Peptide Assay Kit, Product No. 23275)
- Vacuum centrifuge (e.g., Speedvac)
- Mass spectrometer with nano-flow liquid chromatography (LC) system
- (Optional) 20% formic acid (FA) and 5% hydroxylamine for TMT™ reagent labeling

Procedure summary



Procedure

Note: Use 10-100 µg 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

1. For cultured cells, add 100 µL of Lysis Buffer and 1 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 1 µL Universal Nuclease) per 5 mg of tissue and disrupt with tissue homogenizer until sample is homogenized. Centrifuge tissue lysates at 16,000 × *g* for 10 minutes.
3. For purified proteins, serum, and plasma samples, dilute samples directly in Lysis Solution to 0.1-1 mg/mL. 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 10-100 µg of protein sample into the 1.3 mL Sample Prep Plate and adjust the final volume to 100 µL with Lysis Solution.

   Note: Alternatively, samples can be prepared in 2 mL low protein binding tubes (Product No. 88379).
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. Seal the plate with sealing tape and incubate sample at 95°C using heat block for 10 minutes or 50°C for 30 minutes to reduce and alkylate the protein sample.
9. After incubation, allow the sample to cool to room temperature.

Digest protein

1. Add 1 mL of Enzyme Reconstitution Solution to 1 vial of Trypsin/Lys-C Protease Mix to prepare 0.1 µg/µL enzyme mix.
2. Add 50 µL of the reconstituted enzyme solution to the reduced and alkylated protein sample solution.

   Note: For lower sample amounts (< 20 µg), proportionally less enzyme mix can be used. The recommended ratio of sample to enzyme is 1:20, w:w.
3. Incubate with shaking at 37°C for 2-3 hours to digest the protein sample.

   Note: Optional labeling with TMT reagents can be performed before Digestion stop or after peptide clean up.
4. After incubation is complete, add 50 µL of Digestion Stop Solution to acidify the sample and gently mix.

Clean-up peptides

The Peptide Clean-Up Plate is compatible with a centrifuge, vacuum manifold, and positive pressure modes. The protocol below describes the clean up using a centrifuge.

1. Place the Peptide Clean-Up Plate on top of one 2.0 mL collection plate.
2. Transfer the protein digest sample (~300 µL total volume) into the dry Peptide Clean-Up Plate.
3. Centrifuge at 1,000 rpm (250 × *g*) for 10 minutes.
4. Add 300 µL of the Wash Solution A into the Peptide Clean-Up Plate.
5. Centrifuge at 2,000 rpm (1,000 × *g*) for 2 minutes.
6. Add 300 µL of Wash Solution B into the Peptide Clean-Up Plate.
7. Centrifuge at 2,000 rpm (1,000 × *g*) for 2 minutes.
8. Repeat steps 6 and 7 for a total of 2 washes with Wash Solution B.
9. Place the Peptide Clean-Up Plate on top of the new 2.0 mL collection plate.
10. Add 300 µL of the Elution Solution into the Peptide Clean-Up Plate.
11. Centrifuge at 2,000 rpm (1,000 × *g*) for 2 minutes to collect the clean peptide sample.
12. Dry the peptide sample using a vacuum centrifuge.

13. Resuspend the sample in 100 µL of 0.1% formic acid in water for LC-MS analysis.
14. *(Optional)* Assess peptide yield and concentration using a quantitative peptide assay. Adjust the peptide concentration with 0.1% formic acid in water solution for LC-MS column loading.

(Optional) Label protein digest with TMT™ reagent before peptide clean up

Note: 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 10-100 µg digests, add 0.08-0.8 mg of TMT™-labeled reagent (alternatively, 0.1-1 mg of TMTpro™ reagent) dissolved in 40 µL of 100% acetonitrile to each 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. Proceed to the clean-up protocol using the Peptide Clean-Up Plate.

(Optional) Label peptides with TMT™ reagent after peptide clean up

The protocol below describes labeling after peptide clean up as it allows for measuring and normalizing peptide samples for equal mixing.

1. Resuspend 10-100 µg peptide sample in 100 mM TEAB, pH 8.5 or HEPES, pH 8. Verify pH using pH paper.
2. For TMT™-labeled reagent, add 0.08-0.8 mg (for TMTpro™ reagent, add 0.1-1 mg) dissolved in 40 µL of 100% acetonitrile to each buffered peptide sample and incubate for 30-60 minutes at room temperature.
3. Add 8 µL of 5% hydroxylamine to each labeling reaction to quench and incubate for 5 minutes at room temperature.
4. Combine equal amounts of each labeled sample into 1 tube.
5. Acidify sample by adding 5% TFA until pH < 3. Verify pH using pH paper.
6. Desalt combined peptide samples using Pierce™ Peptide Desalting Spin Columns (Catalog No. 89852) or equivalent.

Troubleshooting

Observation	Possible cause	Recommended action
High viscosity sample after lysis.	Universal Nuclease was not added.	Add 1 µL of Universal Nuclease per 100 µL 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™ Maxi MS Sample Prep Kit	A45734
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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