EasyPep™ 96 Micro MS Sample Prep Kit

Catalog Numbers A57864

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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.

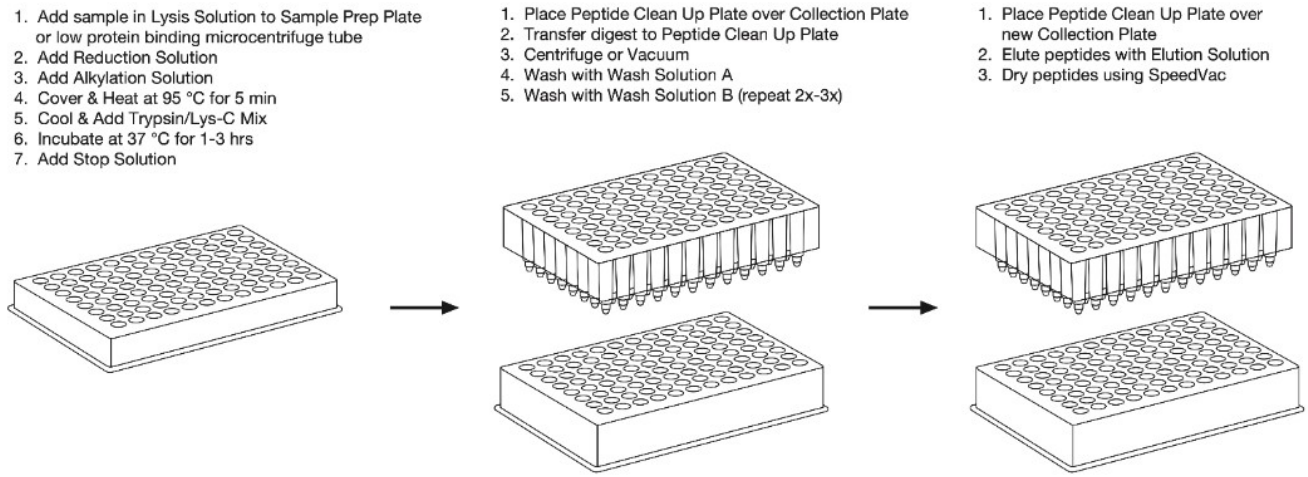
Product description

The Thermo Scientific™ EasyPep™ 96 Micro MS Sample Prep Kit enables efficient and reproducible processing of cultured mammalian cells 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 1-10 µg with a 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 Da), 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, and high pH reversed-phase fractionation with TMT/TMTpro pooled samples.

Contents

Product	Cat. No.	Contents	Storage
EasyPep™ 96 Micro MS Sample Prep Kit	A57864	Kit sufficient for 96 preparations of 1-10 µg Contents: Lysis Solution, 5 mL Universal Nuclease, 25 kU Reduction Solution, 1 mL Alkylation Solution, 1 mL Enzyme Reconstitution Solution, 1 mL Pierce™ Trypsin/Lys-C Protease Mix, MS Grade, 1 × 100 µg Digestion Stop Solution, 1 mL Peptide Clean-Up Plate, 1 each Wash and Elution Plate, 1 each Sample Prep Plate, 1 each Wash Solution A, 40 mL Wash Solution B, 2 × 27 mL Elution Solution, 20 mL PCR Plate Lids, 8 x 12	Store at 4°C. Enzyme components can be stored at -20°C.

Procedure summary



Additional information

- Warm the Lysis Solution to room temperature before use. Store solutions at 4°C.
- Addition of protease inhibitor cocktails containing EDTA to Lysis Solution are **NOT** recommended as these reagents inhibit 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 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)
- Vacuum centrifuge (e.g., Speedvac)
- Mass spectrometer with nano-flow liquid chromatography (LC) system

Procedure

Note: Use 1-10 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 for 10-15 cycles until sample viscosity is reduced. Centrifugation of cultured cell lysates is typically not required after aspiration using pipet.

   Note: For limiting cell number or tissue samples, use an appropriate volume of lysis buffer to achieve a final concentration of 0.1-1 µg/µL in a final volume of 10 µL.
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, depleted and non-depleted plasma, and serum samples, dilute samples directly in Lysis Solution to 0.1-1 µg/µL with a final volume of 10 µL.

   Note: The concentration of 1 µL of plasma is around 60-70 µg. 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 1-10 µg of protein sample into the 0.2 mL Sample Prep Plate and adjust the final volume to 10 µL with Lysis Solution.

   Note: Alternatively, samples can be prepared in 1.5 mL or other low protein binding tubes (Product No. 90410).
6. Add 5 µL of Reduction Solution to the sample and gently mix.
7. Add 5 µL of Alkylation Solution to the sample and gently mix.
8. Seal the plate with PCR plate lids and incubate the sample at 95°C using a heat block for 5 minutes to reduce and alkylate the protein sample.
9. After incubation, allow the sample to cool to room temperature.
10. Briefly centrifuge the plate or tube before digestion.

Digest protein

1. Add 500 µL of Enzyme Reconstitution Solution to 1 vial of Trypsin/Lys-C Protease Mix to prepare 0.2 µg/µL enzyme mix.
2. Add 5 µL of the reconstituted enzyme solution to the reduced and alkylated protein sample solution.

   Note: Aliquot any unused enzyme and store at -20°C for future use.
3. Incubate with shaking at 37°C for 1-3 hours to digest the protein sample.

   Note: Optional labeling with TMT reagents can be performed before the Digestion stop or after peptide clean up.
4. After incubation is complete, add 5 µL of Digestion Stop Solution to the sample and gently mix.

Clean-up peptides

The Peptide Clean-Up Plate is compatible with a centrifuge and vacuum manifold. 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 (~30 µL total volume) into the dry Peptide Clean-Up Plate.
3. Centrifuge at 1,000 rpm for 3 minutes.

   Note: If the samples do not completely flow through after the first spin, centrifuge at 1,500 rpm for 1 minute.
4. Add 100 µL of the Wash Solution A into the plate.
5. Centrifuge at 2,000 rpm for 2 minutes.
6. Add 100 µL of Wash Solution B into the plate.
7. Centrifuge at 2,000 rpm for 2 minutes.

8. Repeat steps 6 and 7 for a total of 2 washes with Wash Solution B.

   Note: For lower sample amounts (< 5 µg) or TMT/TMTpro labeled samples, perform a total of 3 washes with Wash Solution B.
9. Place the Peptide Clean-Up Plate on top of the other 1.0 mL collection plate.
10. Add 100 µL of the Elution Solution into the plate.
11. Centrifuge at 2,000 rpm for 2 minutes to collect the clean peptide sample.
12. Dry the peptide sample using a vacuum centrifuge.
13. Resuspend the sample in 10-20 µL of 0.1% formic acid in water for LC-MS analysis.

    Note: The peptide recovery is around 40-50% of the initial protein digest. Reconstitute the samples accordingly for LC-MS column loading.

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

Note: The protocol below describes labeling the samples before peptide clean up. For 1-5 µg digests, use at least 1:16, w:w, sample to TMT™ reagent and 1:20, w:w, sample to TMTpro™ reagent. For 6-10 µg, use 1:4 or 1:8, w:w, sample to TMT™ reagent and 1:5 or 1:10, w:w, sample to TMTpro™ reagent.

1. Dissolve TMT™ reagent and TMTpro™ reagent in 100% acetonitrile according to the TMT/TMTpro instruction manual.
2. Add appropriate volume of TMT™ or TMTpro™ reagent to each peptide sample according to the recommended sample-to-tag ratio.
3. Incubate for 30-60 minutes at room temperature.
4. Add 5 µL of 0.5% hydroxylamine, 5% 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.
5. 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 1-10 µg peptide sample in 10 µL of 100 mM TEAB, pH 8.5 or HEPES, pH 8. Verify pH using pH paper.
2. Dissolve TMT™ reagent and TMTpro™ reagent in 100% acetonitrile according to the TMT/TMTpro instruction manual.
3. Add appropriate volume of TMT™ or TMTpro™ reagent to each peptide sample according to the recommended sample to tag ratio.
4. Incubate for 30-60 minutes at room temperature.
5. Add 5 µL of 0.5% hydroxylamine to each labeling reaction to quench and incubate for 5 minutes at room temperature
6. Combine equal amounts of each labeled sample into 1 tube.
7. Acidify the sample by adding 5% TFA until pH < 3. Verify pH using pH paper.
8. Desalt combined peptide samples using Pierce™ Peptide Desalting Spin Columns (Catalog No. 89852), Pierce C18 spin columns, stage tips, 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 95°C for 5 minutes.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0029376

Revision	Date	Description
A.0	18 April 2023	Initial release.

The information in this guide is subject to change without notice.