# EasyPep™ Mini MS Sample Prep Kit

**Catalog Numbers** A40006

**Doc. Part No.** 2162714 **Pub. No.** MAN0018079 **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

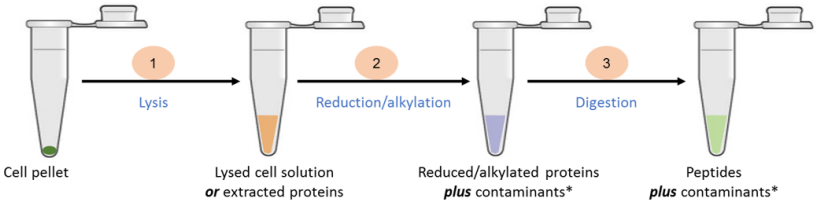
The Thermo Scientific™ EasyPep™ Mini MS Sample Prep Kit enables efficient and reproducible processing of cultured mammalian cells and tissues for proteomic mass spectrometry (MS) analysis. The kit contains pre-formulated buffers, MS-grade enzyme mix, peptide clean-up columns, and an optimized protocol to generate MS-compatible peptide samples in less than 3 hours. The kit is optimized to process 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 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™ reagent) labeling, phosphopeptide enrichment or high pH reversed-phase fractionation.

## Contents

| Product | Cat. No. | Contents | Storage |
|---|---|---|---|
| EasyPep™ Mini MS Sample Prep Kit | A40006 | Kit sufficient for 20 preparations of 10-100 µg<br>Contents:<br>Lysis Solution, 5 mL<br>Universal Nuclease, 5 ku<br>Reduction Solution, 1 mL<br>Alkylation Solution, 1 mL<br>Pierce™ Trypsin/Lys-C Protease Mix, MS Grade, 2 × 100 µg<br>Digestion Stop Solution, 1 mL<br>Peptide Clean-Up Columns, 20 each<br>Wash Solution A, 6 mL<br>Wash Solution B, 12 mL<br>Elution Solution, 6 mL<br>Low Protein Binding Collection Tubes, 2 mL, 40 each | Store at 4°C.<br>Enzyme components can be stored at -20°C. |

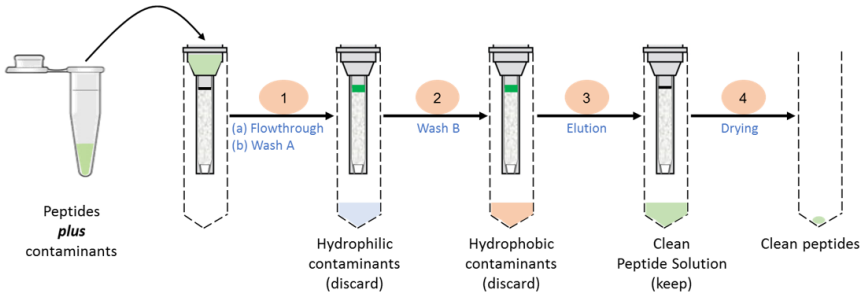
## Procedure summary

Stage 1: Chemical & Enzymatic Sample Processing




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

Stage 2: Peptide Clean-Up



## 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.
- 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 columns is required to remove contaminants and enzymes before LC-MS analysis.

## Materials required but not supplied

- *(Optional)* Tissue homogenizer
- Heat block or thermo mixer
- Protein assay kit (e.g., Thermo Scientific™ Pierce™ BCA Protein Assay Kit, Cat. No. 23227)
- Mass spectrometer with nano-flow liquid chromatography (LC) system

## 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 $1 \times 10^6$ 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 (Cat. No. 23227) or Pierce™ Rapid Gold BCA Protein Assay Kit (Cat. No. A53226).
5. Transfer 10-100 µg of protein sample into a new 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 to reduce and alkylate the protein sample.
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 50 µL of the reconstituted enzyme solution to the reduced and alkylated protein sample solution.

   **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.

   **Note:** At this point, the protein digest can be labeled with TMT™ reagents before peptide clean-up. If you are performing this protocol, proceed directly to "Label peptides with TMT™ reagent before peptide clean-up" on page 3.
4. After incubation is completed, add 50 µL of Digestion Stop Solution to the sample and gently mix.

### Clean-up peptides

1. Remove the white cap at the bottom of the Peptide Clean-up column, loosen the green top cap, and place into a 2 mL microcentrifuge tube.
2. Centrifuge at 3,000 × *g* for 2 minutes to remove all liquid from the column. Discard the flowthrough.
3. Transfer the protein digest sample (~300 µL total volume) into the dry Peptide Clean-up column.
4. Centrifuge at 1,500 × *g* for 2 minutes. Discard the flowthrough.
5. Add 300 µL of the Wash Solution A into the column.
6. Centrifuge at 1,500 × *g* for 2 minutes. Discard the flowthrough.
7. Wash sample twice with Wash Solution B.
   a. Add 300 µL of Wash Solution B into the column.
   b. Centrifuge at 1,500 × *g* for 2 minutes. Discard the flowthrough.
   c. Repeat steps one time.
8. Transfer the Peptide Clean-up column into a new 2 mL microcentrifuge tube.
9. Add 300 µL of the Elution Solution into the column.
10. Centrifuge at 1,500 × *g* for 2 minutes to collect the clean peptide sample.
11. Dry the peptide sample using a vacuum centrifuge.
12. Resuspend the sample in 100 µL of 0.1% formic acid in water for LC-MS analysis.
13. *(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.

### (Optional) Label peptides with TMT™ reagent

The Tandem Mass Tag™ (TMT™) is used in isobaric labeling as a method to quantify relative differences in protein samples. TMT™ labeling can be performed either immediately after protein digestion (i.e., before peptide clean-up) or after peptide clean-up. Labeling peptides with TMT™ reagents after clean up allows for measuring and normalizing peptide samples for equal mixing.

#### Label peptides with TMT™ reagent before peptide clean-up

1. Add 40 µL of TMT™ reagent dissolved in 100% acetonitrile to each buffered peptide sample and incubate for 30-60 minutes at room temperature.
   - For TMT™ label reagent, use 0.08 to 0.8 mg of label reagent for 10-100 µg of protein digest.
   - For TMTpro™ label reagent, use 0.1 to 1 mg of label reagent for 10-100 µg of protein digest.
2. Add 50 µL of 5% hydroxylamine, 20% formic acid solution to each labeling reaction to quench and acidify.

   **Note:** The 5% hydroxylamine, 20% formic acid solution replaces the Digestion Stop Solution used in step 4 of the label-free sample preparation protocol (see "Digest protein" on page 2).
3. Verify pH < 4 using pH paper.
4. Proceed to "Clean-up peptides" on page 2.

#### Label peptides with TMT™ reagent after peptide clean-up

1. Resuspend 10-100 µg peptide sample in 100 mM TEAB, pH 8.5 or HEPES, pH 8. Verify pH using pH paper.
2. Add 40 µL of TMT™ reagent dissolved in 100% acetonitrile to each buffered peptide sample and incubate for 30-60 minutes at room temperature.
   - For TMT™ label reagent, use 0.08 to 0.8 mg of label reagent for 10-100 µg of peptide sample.
   - For TMTpro™ label reagent, use 0.1 to 1 mg of label reagent for 10-100 µg of peptide sample.
3. Add 8 µL of 5% hydroxylamine to each labeling reaction to quench and incubate for 15 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, Cat. 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. |
| | 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. |
| Overestimation of peptide yield using peptide assays. | Incomplete removal of elution buffer during speedvac. | Speedvac eluted samples completely. |
| | | Use Pierce™ Peptide Desalting Spin Columns (Cat. No. 89852) to remove excess buffer or TMT™ reagents. |

## Related products

| Product | Cat. No. |
|---|---|
| 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 |

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