Peptide Clean-Up Plate

Catalog Numbers A57865

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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™ Peptide Clean-Up Plate is a ready-to-use plate that enables efficient contaminant removal from 10 µg to 100 µg peptide samples following enzymatic digestion. The resin contained in each well provides excellent binding and recovery characteristics of peptide samples in preparation for mass spectrometry and other methods. The plate format allows the processing of multiple samples (1-96) in parallel in less than 30 minutes.

Contents

Product	Cat. No.	Contents	Storage
Peptide Clean-Up Plate	A57865	Plate sufficient for clean-up of 96 preparations of 10-100 µg peptides Contents: Collection Plate, 2 mL, 2 each Peptide Clean-Up Plate, 1 each	Store at room temperature.

Additional information

- Use of a 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 room temperature or 4°C if being used multiple times to prevent contamination.
- Compatible with Urea and SDS- based extraction buffers (do not exceed 0.1% SDS).
- Urea lysis buffer should be prepared fresh prior to each experiment and used at room temperature. Placing the urea lysis buffer on ice will cause the urea to precipitate out of the solution.
- Avoid temperatures higher than 60°C for the samples with urea-based lysis buffer to prevent carbamylation of lysines and protein N-termini.
- Prepare solutions with MS or equivalent-grade water.
- The iodoacetamide solution should be prepared fresh prior to each experiment. After weighing the powder, store it in the dark and add water only immediately before use.

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

Procedure

The protocol below describes the general protocol for the sample prep. Results will vary based on the protein extraction buffer formulation, wash, and elution conditions. 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. Lyse the cells by adding five cell-pellet volumes of urea or SDS-based lysis Buffer (i.e., 100 µL of lysis buffer for a 20 µL cell pellet). During lysis, the sample becomes viscous due to DNA released from the cells. Sonicate the lysate using a micro tip sonicator or add 25 units of Universal Nuclease to 1mL of cell lysate and incubate at room temperature for 15 minutes. Repeat pipetting until sample viscosity is reduced.
2. Clear the lysate by centrifugation in a microcentrifuge at 16,000 x *g* for 5-10 minutes at 4°C.
3. Carefully separate the supernatant and transfer into a new tube.
4. For tissue samples, SDS-based lysis buffer is recommended. Add 100 µL of Lysis Solution (containing 1 µL Universal Nuclease) per 5 mg of tissue and disrupt with tissue homogenizer until the sample is homogenized. Centrifuge tissue lysates at 16,000 × *g* for 10 minutes.

5. For 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 plasma samples, addition of Universal Nuclease is **not** required.
6. 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).
7. Transfer 10-100 µg of protein sample into the sample prep plate.

   Note: Alternatively, samples can be prepared in 2 mL low protein binding tubes (Product No. 88379).
8. Reduce the proteins by adding 1M DTT solution with a final concentration of 10mM DTT, mix well, and incubate at 37°C for 30 minutes for urea containing lysis buffer and 95°C for 5-10 minutes for SDS containing lysis buffer.
9. After incubation, allow the sample to cool to room temperature.
10. Alkylate the proteins by adding 0.5M iodoacetamide solution with a final concentration of 40mM IAA in the sample, mix well and incubate at room temperature for 30 minutes in the dark.
11. Quench unreacted iodoacetamide by adding 1M DTT to a final concentration of 10mM and incubate at room temperature for 15 minutes.
12. For samples containing 8M urea in the lysis buffer, dilute the samples with 25mM Tris pH 8 to reduce the concentration of urea in the sample to ≤1M.

Digest protein

1. *(Optional)* Add Lys-C and incubate with shaking at 37°C for 2 hours (1:50 w/w).
2. Add trypsin (1:50 w/w) and incubate with shaking at 37°C for overnight to digest the protein sample.

   Note: Alternatively, Trypsin/Lys-C protease mixture can be used at a 1:50 enzyme-to-protein ratio to digest the protein sample. Higher enzyme-to-protein ratios can be used to digest the sample in a few hours. Other enzymes can be utilized but have not been tested.
3. After incubation is complete, acidify the digested peptides with Formic acid or TFA to pH ~ 3.

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.

Note: Use the same washing and elution strategy for the vacuum manifold and positive pressure.

1. Place the Peptide Clean-Up Plate on top of one 2.0 mL collection plate.
2. Transfer the acidified protein digest sample into the dry Peptide Clean-up plate.

   Note: Acidify the sample with Formic acid or TFA before loading onto the peptide clean-up plate.
3. Centrifuge at 1,000 rpm for 10 minutes.

   Note: Check each well of the Clean-Up Plate to make sure all the liquid has passed through. Centrifuge for additional time if needed.
4. Add 300 µL of 5% acetonitrile, 0.2% formic acid, 95% water into the plate.
5. Centrifuge at 2,000 rpm for 2 minutes.
6. Add 300 µL of 100% acetonitrile into the plate.
7. Centrifuge at 2,000 rpm for 2 minutes.
8. Place the Peptide Clean-Up Plate on top of new 2.0 mL collection plate.
9. Add 300 µL of 40% acetonitrile, 5% ammonium hydroxide, 55% water into the plate.
10. Centrifuge at 2,000 rpm for 2 minutes to collect the clean peptide sample.
11. Dry the peptide sample using a vacuum centrifuge.
12. Resuspend the sample in 0.1% formic acid in water to desired concentration for LC-MS analysis.
13. *(Optional)* Assess peptide yield and concentration. Adjust the peptide concentration with 0.1% formic acid in water solution for LC-MS column loading.

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.
	Lysate was not sonicated.	Sonicate the lysate on a micro-tip sonicator.
Incomplete digestion.	Inactive enzyme.	Store any remaining enzyme solution in single-use volumes at -80°C.
		Cool samples after reduction/alkylation to room temperature before addition of enzyme/protease mix.
Low protein yield.	Insufficient cells.	Increase the number of cells used for lysis.
Over-alkylation.	Alkylation occurred for too long.	Alkylate the reduced protein protected from light for 30 minutes at room temperature and quench unreacted iodoacetamide by adding DTT (10 mM final concentration).
Low peptide yield.	Sample not acidified before clean-up.	Add formic acid or TFA to acidify the digested sample.

Limited product warranty

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Thermo Fisher Scientific | 3747 N. Meridian Road | Rockford, Illinois 61101 USA

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

Revision history: Pub. No. MAN0029375

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

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