INSTRUCTIONS

Mem-PER™ Plus Membrane Protein Extraction Kit

89842

2519.1

Number	Description
89842	**Mem-PER Plus Membrane Protein Extraction Kit**, contains sufficient lysis and extraction reagents for approximately 50 mammalian cell pellet fractions containing 5×10^6 cells each or 25 tissue samples containing 20-40mg of tissue

Kit Contents:

Cell Wash Solution, 225mL; store at 4°C

Solubilization Buffer, 25mL; store at 4°C

Permeabilization Buffer, 50mL; store at -20°C

Storage: Upon receipt store Cell Wash Solution and Solubilization Buffer at 4°C. Aliquot and store Permeabilization Buffer at -20°C to avoid freeze/thaws. Product is shipped on ice.

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Introduction

The Thermo Scientific™ Mem-PER™ Plus Membrane Protein Extraction Kit is used for the enrichment of integral membrane proteins and membrane-associated proteins from cultured mammalian cells or tissue. The kit uses a mild detergent-based, selective extraction protocol, which eliminates the hassle of phase separation based on hydrophobicity, allowing better reproducibility and higher throughput.

The cells are permeabilized with a mild detergent to allow the release of soluble cytosolic proteins. A second detergent then solubilizes membrane proteins. Extraction efficiencies will vary depending on the number of times the integral membrane protein(s) of interest spans the lipid bilayer. Membrane proteins with at least 1-2 transmembrane domains are typically extracted with an efficiency of up to 90%. Cross-contamination of cytosolic proteins into the membrane fraction is usually less than 10%.

Important Product Information

- For optimal results, include protease and phosphatase inhibitors (e.g., Product No. 78440) in the Permeabilization and Solubilization Buffers.
- To directly quantify proteins in the cytosolic and/or membrane fraction, use a protein assay such as the Thermo Scientific™ BCA Protein Assay (Product No. 23225), Micro BCA™ Protein Assay (Product No. 23235) or Pierce™ 660nm Protein Assay (Product No. 22660).

- Both fractions are compatible with SDS-PAGE. Use the Thermo Scientific™ Pierce™ SDS-PAGE Sample Prep Kit (Product No. 89888) if the protein of interest is in low abundance and a large volume is required for adequate detection.
- If the detergent in the membrane fraction interferes with downstream applications, use Thermo Scientific™ Pierce™ Detergent Removal Spin Columns (Product No. 87776-7) to decrease the amount of detergent in the sample. However, the sample:resin ratio will need to be empirically determined since removing too much detergent may result in protein precipitation.

Additional Material Required

- Protease and phosphatase inhibitors (e.g., Product No. 78440)
- Temperature-controlled mixer
- Cell scraper to remove adherent cells from plates
- 5mL microcentrifuge tubes
- For soft tissues, a 2mL Dounce Tissue Grinder (e.g., Kontes or Wheaton Tenbroeck) is required.
- For hard tissues, a hand-held homogenizer for 0.5-1.5mL samples (e.g., Brinkmann™ Polytron™ PT-1200CL Homogenizer)

Procedure for Membrane Protein Extraction from Different Sample Types

Choose the appropriate protocol for the sample type used.

Protocol 1: Adherent Mammalian Cells

1. Resuspend 5×10^6 cells in the growth media by scraping the cells off the surface of the plate with a cell scraper. Centrifuge harvested cell suspension at $300 \times g$ for 5 minutes.
2. Wash cell pellet with 3mL of Cell Wash Solution and centrifuge at $300 \times g$ for 5 minutes.
3. Carefully remove and discard the supernatant. Resuspend the cells in 1.5mL of Cell Wash Solution and transfer to a 2mL centrifuge tube. Centrifuge at $300 \times g$ for 5 minutes and discard supernatant.
4. Add 0.75mL of Permeabilization Buffer to the cell pellet. Vortex briefly to obtain a homogeneous cell suspension. Incubate 10 minutes at 4°C with constant mixing.
5. Centrifuge permeabilized cells for 15 minutes at $16,000 \times g$. Carefully remove the supernatant containing cytosolic proteins and transfer to a new tube.
6. Add 0.5mL of Solubilization Buffer to the pellet and resuspend by pipetting up and down. Incubate tubes at 4°C for 30 minutes with constant mixing.
7. Centrifuge tubes at $16,000 \times g$ for 15 minutes at 4°C. Transfer supernatant containing solubilized membrane and membrane-associated proteins to a new tube.
8. Proceed to downstream application. Immediately use cytosolic and membrane fractions stored on ice or store aliquots at -80°C for future use.

Protocol 2: Suspension Mammalian Cells

1. Harvest 5×10^6 cells by centrifugation at $300 \times g$ for 5 minutes. Wash harvested cell pellet with 3mL of Cell Wash Solution and centrifuge at $300 \times g$ for 5 minutes.
2. Carefully remove and discard the supernatant. Resuspend the cells in 1.5mL of Cell Wash Solution and transfer to a 2mL centrifuge tube. Centrifuge at $300 \times g$ for 5 minutes and discard supernatant.
3. Add 0.75mL of Permeabilization Buffer to the cell pellet. Vortex briefly to obtain a homogeneous cell suspension. Incubate 10 minutes at 4^oC with constant mixing.
4. Centrifuge permeabilized cells for 15 minutes at $16,000 \times g$. Carefully remove the supernatant containing cytoslic proteins and transfer to a new tube.

5. Add 0.5mL of Solubilization Buffer to the pellet and resuspend by pipetting up and down. Incubate tubes at 4°C for 30 minutes with constant mixing.
6. Centrifuge tubes at 16,000 × *g* for 15 minutes at 4°C. Transfer supernatant containing solubilized membrane and membrane-associated proteins to a new tube.
7. Proceed to downstream application. Immediately use cytosolic and membrane fractions stored on ice or store aliquots at -80°C for future use.

Protocol 3: Soft Tissue

1. Place 20-40mg of soft tissue in a 5mL microcentrifuge tube. Add 4mL of Cell Wash Solution to the tissue, vortex briefly and discard wash.
2. Transfer to a 2mL tissue grinder and cut the tissue into small pieces with a pair of scissors. Add 1mL of permeabilization buffer to the tissue and homogenize until an even suspension is obtained (6-10 strokes).
3. Add 1mL of Permeabilization Buffer and transfer homogenate to a new tube, incubating for 10 minutes at 4°C with constant mixing.

   Optional: Remove non-homogenized tissue using the protocol provided with the Thermo Scientific™ Pierce™ Tissue Strainers (Product No. 87791) before pelleting permeabilized cells.
4. Centrifuge at 16,000 × *g* for 15 minutes at 4°C to pellet permeabilized cells. Carefully remove the supernatant containing cytosolic proteins and transfer to a new tube.
5. Resuspend the pellet in 1mL of Solubilization Buffer. Pipette up and down to obtain a homogeneous suspension. Incubate 30 minutes at 4°C with constant mixing.
6. Centrifuge tubes at 16,000 × *g* for 15 minutes at 4°C. Transfer supernatant containing solubilized membrane and membrane-associated proteins to a new tube.
7. Proceed to downstream application. Immediately use cytosolic and membrane fractions stored on ice or store aliquots at -80°C for future use.

Protocol 4: Hard Tissue

1. Place 20-40mg of hard tissue in a 5mL microcentrifuge tube. Add 4mL of Cell Wash Solution to the tissue, vortex briefly and discard the wash.
2. Add 1mL of Permeabilization Buffer to the tissue and cut the tissue into small pieces with a pair of scissors.
3. Homogenize minced tissue with a hand-held Polytron Homogenizer. Use a low setting (to prevent foaming) until an even suspension is obtained.
4. Add an additional 1mL of Permeabilization Buffer to the homogenate and incubate for 10 minutes on ice with constant mixing.

   Optional: Remove non-homogenized tissue using protocol provided with the Pierce Tissue Strainers before pelleting permeabilized cells.
5. Centrifuge at 16,000 × *g* for 15 minutes at 4°C to pellet permeabilized cells. Carefully remove the supernatant containing cytosolic proteins and transfer to a new tube.
6. Resuspend the pellet in 1mL of Solubilization Buffer. Pipette up and down to obtain a homogeneous suspension. Incubate 30 minutes at 4°C with constant mixing.
7. Centrifuge tubes at 16,000 × *g* for 15 minutes at 4°C. Transfer supernatant containing solubilized membrane and membrane-associated proteins to a new tube.
8. Proceed to downstream application. Immediately use cytosolic and membrane fractions stored on ice or store aliquots at -80°C for future use.

Troubleshooting

Problem	Possible Cause	Solution
Contamination of membrane fraction with cytosolic proteins	Cells were not permeabilized	Increase incubation time or strokes of homogenization
	Permeabilization Buffer was improperly stored	Store Permeabilization Buffer at -20°C
	Incomplete removal of permeabilized cell supernatant	Ensure complete removal of cytosolic extract
Low membrane protein yield	Membranes were solubilized by Permeabilization Buffer	Decrease incubation time or strokes
	Incomplete membrane protein isolation	Increase incubation time
Low overall protein yield	Not enough cells/tissue	Increase cell number or amount of starting tissue (mg)

Related Thermo Scientific Products

87785 **Halt™ Protease Inhibitor Cocktail, EDTA-Free (100X),** 1mL

87786 **Halt Protease Inhibitor Cocktail (100X),** 1mL

78441 **Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-Free (100X),** 1mL

78440 **Halt Protease and Phosphatase Inhibitor Cocktail (100X),** 1mL

78420 **Halt Phosphatase Inhibitor Cocktail (100X),** 1mL

88665-9 **Pierce Protease Inhibitor Tablets**

23225 **BCA Protein Assay Kit**

89888 **Pierce SDS-PAGE Sample Prep Kit**

87777 **Pierce Detergent Removal Spin Columns,** 0.5mL

78840 **Subcellular Protein Fractionation Kit for Cultured Cells**

87790 **Subcellular Protein Fractionation Kit for Tissue**

87791 **Pierce Tissue Strainers, 250µm, 2.5mL**, 50 units

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

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