INSTRUCTIONS

Mem-PER™ Eukaryotic Membrane Protein Extraction Reagent Kit

89826

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Number	Description
89826	**Mem-PER Eukaryotic Membrane Protein Extraction Reagent Kit**, contains sufficient lysis and extraction reagents for approximately 60-80 mammalian cell pellet fractions containing 5×10^6 cells each, 15mg of wet yeast cell paste, or 20mg of tissue

Kit Contents:

Mem-PER Reagent A, 10mL

Mem-PER Reagent B, 25mL

Mem-PER Reagent C, 40mL

Storage: Upon receipt store Reagent A at room temperature. Store Reagent B and Reagent C at 4°C. Product is shipped at ambient temperature.

Note: The ambient shipping conditions may cause Reagent C to appear cloudy upon receipt. Storage at 4°C will clarify the solution. Keep Reagent C at 4°C or on ice at all times.

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Introduction

The Thermo Scientific™ Mem-PER™ Eukaryotic Membrane Protein Extraction Reagent Kit is for the enrichment of integral membrane proteins from cultured mammalian or yeast cells or from mammalian tissue using a mild detergent-based protocol. The cells are first lysed with a detergent, after which a second detergent is added to solubilize the membrane proteins. The cocktail is incubated at 37°C to separate the hydrophobic proteins from the hydrophilic proteins through phase partitioning. Extraction efficiencies will vary depending on the number of times the integral membrane protein(s) of interest spans the lipid bilayer. Membrane proteins with up to four transmembrane domains are typically extracted with an efficiency of up to 90%. Cross-contamination of cytosolic proteins into the membrane fraction is typically < 10%.

Important Product Information

- For optimal results, include protease inhibitors (e.g., Product No. 78415) in the Mem-PER Reagents.
- Perform 37°C incubation step in a water bath. Incubator ovens do not heat evenly enough for this application.
- Do not exceed 10 samples in one extraction procedure because rapid resolubilization of the hydrophobic phase into the hydrophilic phase occurs at room temperature.
- For downstream processing of samples, refer to the Preparation of Samples for Downstream Applications Section following the main protocols.

Additional Material Required

- Protease inhibitors (e.g., Product No. 78415)
- For soft tissues, a 2mL Dounce Tissue Grinder, such as Kontes or Wheaton Tenbroeck, is required.
- For hard tissues, a hand-held homogenizer for 0.5 to 1.5mL samples, such as the Brinkmann™ Polytron™ PT-1200CL, is required.
- Tris Buffered Saline (TBS; 0.025M Tris, 0.15M NaCl; pH 7.2; Product No. 28376) containing protease inhibitors
- Water bath equilibrated to 37°C. Do not use an incubator oven, which will not heat evenly enough for this application.

Procedure for Membrane Protein Extraction from Different Sample Types

Following are four protocols: Protocol 1 is for the extraction of membrane proteins from mammalian cells; Protocol 2 is for the extraction of membrane proteins from yeast cells: Protocol 3 is for the extraction of membrane proteins from soft tissues; and Protocol 4 is for the extraction of membrane proteins from hard tissues. (See the Additional Information Section for a schematic of the mammalian cells and tissue protocols.)

Note: Additional protein recovery may be recovered with a second extraction; see protocol at the end of this section.

In protocol 1: Mammalian Cells, note that protease inhibitors should be added to Reagent A in Step 3. and to the Diluted Reagent C in step 6 prior to adding these to sample.

In all other protocols, note that protease inhibitor should be added to Reagent A in Step 4. and to the Diluted Reagent C in step 7 prior to adding these to sample.

Protocol 1: Mammalian Cells

Note: For optimal results, include protease inhibitors (e.g., Product No. 78415) in the Mem-PER Reagents A, B or C.

1. Isolate 5×10^6 cells per sample by centrifuging harvested cell suspensions at $850 \times g$ for 2 minutes. Pellet cells (washed in PBS) in 1.7mL microcentrifuge tubes.
2. Carefully remove and discard the supernatant.
3. Add 150µL of Reagent A to the cell pellet. Pipette up and down to obtain a homogeneous cell suspension. Incubate 10 minutes at room temperature with occasional vortexing.

   Note: White, flocculent debris appears upon addition of Reagent A.

   Note: To check the cell lysis efficiency, spot 5µL of cell lysate onto a glass slide, add coverslip and view under a light microscope. Compare with 5µL of the same number of intact cells in 150µL of phosphate-buffered saline (PBS) or Tris-buffered saline (TBS).
4. Place lysed cells on ice.
5. Dilute 2 parts Reagent C with 1 part Reagent B, making sufficient mixture for each sample to receive 450µL (e.g., for 10 extractions, combine 3.33mL of Reagent C with 1.67mL of Reagent B). Keep Reagents B and C at 4°C or on ice at all times.

6. Add 450µL of diluted Reagent C to each tube of lysed cells and vortex. Incubate tubes on ice for 30 minutes, vortexing every 5 minutes.
7. Centrifuge tubes at 10,000 × *g* for 3 minutes at 4°C. Transfer supernatant to new tubes and incubate 10 minutes in 37°C water bath to separate the membrane protein fraction. To enhance phase separation, increase incubation to 20 minutes.
8. Centrifuge tubes at room temperature for 2 minutes at 10,000 × *g* to isolate the hydrophobic fraction (i.e., the fraction containing membrane protein of interest) from the hydrophilic fraction.
9. Carefully remove the hydrophilic phase (top layer) from the hydrophobic protein phase (bottom layer) and save in a new tube. Perform phase separations as quickly as possible because the interface between the layers slowly disappears at room temperature.
10. Place the separated fractions on ice. The majority of membrane protein will be in the lower hydrophobic fraction, which can be used for membrane protein analysis.

Protocol 2: Yeast Cells

Note: For optimal results, include protease inhibitors (e.g., Product No. 78415) in the Mem-PER Reagents A, B or C.

1. Prepare approximately 150mg of 405-600µm acid-washed glass beads
2. Harvest yeast cells in the exponential growth phase at a density of 1-5 × 10^7 cells/mL with an OD_{600} = 0.3-1.7. Use approximately, 15mg of wet cell paste per assay.
3. Pellet cells by pulse centrifugation and carefully remove the supernatant.
4. Resuspend cells in 80µL of Reagent A. Pipette up and down to obtain a homogeneous cell suspension.
5. Add the acid-washed glass beads to the cell suspension and vortex for 10 minutes to lyse cells. Pellet the beads by pulse centrifugation. Transfer the cell suspension into a new microcentrifuge tube and keep on ice.
6. Dilute 2 parts Reagent C with 1 part Reagent B, making sufficient mixture for each sample to receive 720µL (e.g., for 10 extractions, combine 4.8mL of Reagent C with 2.4mL of Reagent B). Keep Reagents B and C at 4°C or on ice at all times.
7. Add 720µL of diluted Reagent C into the tube containing the glass beads and briefly vortex to wash. Perform a pulse spin to gather beads.
8. Transfer wash into tube containing the cell suspension and incubate on ice for 30 minutes, vortexing every five minutes.
9. Centrifuge at 10,000 × *g* for three minutes at 4°C. Transfer supernatant to new tubes and incubate 10 minutes in 37°C water bath to separate the membrane protein fraction. To enhance phase separation, increase incubation to 20 minutes.
10. Centrifuge tubes at room temperature (RT) for two minutes at 10,000 × *g* to isolate the hydrophobic fraction (i.e., the fraction containing the membrane protein of interest) from the hydrophilic fraction.
11. Carefully remove hydrophilic phase (top layer) from the hydrophobic phase (bottom layer) and save in a new tube. Perform phase separations as quickly as possible because the interface between layers slowly disappears at RT.
12. Place the separated fractions on ice. The majority of membrane proteins will be in the lower hydrophobic fraction, which can be used for membrane protein analysis.

Protocol 3: Soft Tissue

Note: For optimal results, include protease inhibitors (e.g., Product No. 78415) in the Mem-PER Reagents A, B or C.

1. Place 20mg of soft tissue in a 1.5mL microcentrifuge tube. Add 200µL of TBS to tissue, vortex briefly and discard wash.
2. Transfer rinsed tissue to a 2mL tissue grinder. Add 200µL of TBS to the tissue and homogenize until an even suspension is obtained (~6 to 10 strokes).
3. Transfer homogenate to a new 1.5mL tube and centrifuge at 1000 × *g* for 5 minutes at 4°C.
4. Discard supernatant and resuspend the pellet in 150µL of Reagent A. Pipette up and down to obtain a homogeneous suspension. Incubate 10 minutes at room temperature with occasional vortexing.

   Note: White, flocculent debris appears upon addition of Reagent A.

5. Place lysed cells on ice.
6. Dilute 2 parts Reagent C with 1 part Reagent B, making sufficient mixture for each sample to receive 450µL (e.g., for 10 extractions, combine 3.33mL of Reagent C with 1.67mL of Reagent B). Keep Reagents B and C at 4°C or on ice at all times.
7. Add 450µL of diluted Reagent C to each tube of lysed cells and vortex. Incubate tubes on ice for 30 minutes, vortexing every 5 minutes.
8. Centrifuge tubes at 10,000 × *g* for 3 minutes at 4°C. Transfer supernatant to new tubes and incubate 10 minutes in 37°C water bath to separate the membrane protein fraction. To enhance phase separation, increase incubation to 20 minutes.
9. Centrifuge tubes at room temperature (RT) for 2 minutes at 10,000 × *g* to isolate the hydrophobic fraction (i.e., the fraction containing membrane protein of interest) from the hydrophilic fraction.
10. Carefully remove hydrophilic phase (top layer) from the hydrophobic protein phase (bottom layer) and save in a new tube. Perform phase separations as quickly as possible because the interface between layers slowly disappears at RT.
11. Place the separated fractions on ice. The majority of membrane protein will be in the lower hydrophobic fraction, which can be used for membrane protein analysis.

Protocol 4: Hard Tissue

Note: For optimal results, include protease inhibitors (e.g., Product No. 78415) in the Mem-PER Reagents A, B or C.

1. Place 20mg of hard tissue in a 2.0mL microcentrifuge tube. Add 500µL of TBS to the tissue, vortex briefly and discard the wash.
2. Add 500µL of TBS to the tissue and cut the tissue into small pieces with a clean razor blade.
3. Homogenize minced tissue with a hand-held Polytron®, using a low setting to prevent foaming. Transfer homogenate to a new 1.5mL tube and centrifuge at 1000 × *g* for 5 minutes at 4°C.
4. Discard the supernatant and resuspend the pellet in 150µL Reagent A. Pipette up and down to obtain a homogeneous suspension. Incubate 10 minutes at room temperature with occasional vortexing.

   Note: White, flocculent debris appears upon addition of Reagent A.
5. Place lysed cells on ice.
6. Dilute 2 parts Reagent C with 1 part Reagent B, making sufficient mixture for each sample to receive 450µL (e.g., for 10 extractions, combine 3.33mL of Reagent C with 1.67mL of Reagent B). Keep Reagents B and C at 4°C or on ice at all times.
7. Add 450µL of diluted Reagent C to each tube of lysed cells and vortex. Incubate tubes on ice for 30 minutes, vortexing every 5 minutes.
8. Centrifuge tubes at 10,000 × *g* for 3 minutes at 4°C. Transfer supernatant to new tubes and incubate 10 minutes in 37°C water bath to separate the membrane protein fraction. To enhance phase separation, increase incubation to 20 minutes.
9. Centrifuge tubes at room temperature for 2 minutes at 10,000 × *g* to isolate the hydrophobic fraction (i.e., the fraction containing membrane protein of interest) from the hydrophilic fraction.
10. Carefully remove the hydrophilic phase (top layer) from the hydrophobic protein phase (bottom layer) and save in a new tube. Perform the phase separations as quickly as possible because the interface between the layers slowly disappears at room temperature.
11. Place the separated fractions on ice. The majority of membrane protein will be in the lower hydrophobic fraction, which can be used for membrane protein(s) analysis.

Procedure for Performing a Second Extraction

Although typically not necessary, a second extraction may result in additional protein recovery.

1. Determine volume of the hydrophilic fraction and add an equal volume of undiluted Reagent C to this fraction and vortex.
2. Incubate tubes on ice for 30 minutes, vortexing every 5 minutes during the incubation.

3. Incubate tubes for 10 minutes in a 37°C water bath to separate the membrane protein fraction. To enhance phase separation, increase incubation to 20 minutes.
4. Centrifuge tubes at room temperature for 2 minutes at 10,000 × *g* to isolate the hydrophobic fraction from the hydrophilic fraction.
5. Carefully remove the hydrophilic phase (top layer) from the hydrophobic protein phase (bottom layer) and save in a new tube. Perform phase separations as quickly as possible because the interface between layers slowly disappears at room temperature. Place separated fractions on ice.
6. Combine hydrophobic fraction from the first extraction with the hydrophobic fraction from the second extraction.

Preparation of Samples for Downstream Applications

A. SDS Polyacrylamide Gel Electrophoresis (PAGE)

To determine extraction efficiency, perform SDS-PAGE and identify the protein(s) of interest by Western blotting. Dilute membrane fractions 2- to 5-fold to prevent band and lane distortion caused by high concentrations of detergent. Use Reagent B diluted 4-fold with ultrapure water for dilution and/or normalization. Alternatively, use the Thermo Scientific™ Pierce™ SDS-PAGE Sample Prep Kit (Product No. 89888) to decrease detergent in the membrane fraction. Use this kit if the protein of interest is in low abundance and a large volume is required for adequate detection; for samples prepared using two extractions; or for proteins that migrate at or near the dye front because migrating detergent at the same location causes severe band distortion.

B. Protein Quantitation

To directly quantify proteins in the **hydrophilic** fraction, use a protein assay, such as the Pierce BCA Protein Assay (Product No. 23225), Thermo Scientific™ Micro BCA™ Protein Assay (Product No. 23235), Thermo Scientific™ Coomassie Plus™ (Bradford) Protein Assay (Product No. 23236) or Pierce 660nm Protein Assay (Product No. 22660).

To quantify proteins in the **hydrophobic** fraction, first decrease detergent in the membrane fraction to prevent interference. If performing subsequent SDS-PAGE, we recommend using the Pierce SDS-PAGE Sample Prep Kit (Product No. 89888) followed directly by the Pierce BCA Protein Assay Kit. If performing applications other than SDS-PAGE after quantitation, see Section C below.

C. Other Applications

If the hydrophobic fraction requires decreased detergent for other applications, such as functional studies, immunoprecipitation or quantitation, an alternative option is to use Pierce Detergent Removal Spin Columns (Product No. 87776-7). The sample:resin ratio will need to be determined empirically: If too little detergent is removed, the remaining detergent may interfere with the downstream application. If too much detergent is removed, protein precipitation may occur.

Additional Information

Mem-PER Eukaryotic Membrane Protein Extraction Reagent Kit is for the enrichment of integral membrane proteins using a mild detergent-based protocol. For mammalian cells, a detergent is used to first lyse cells, and then a second detergent is added to solubilize the membrane proteins. The cocktail is incubated at 37°C to separate the hydrophobic proteins from the hydrophilic proteins through phase partitioning (Figure 1). Yeast cells require the use of acid-washed glass beads for cell disruption. Hard and soft tissues also require mechanical means for disruption (Figure 2).

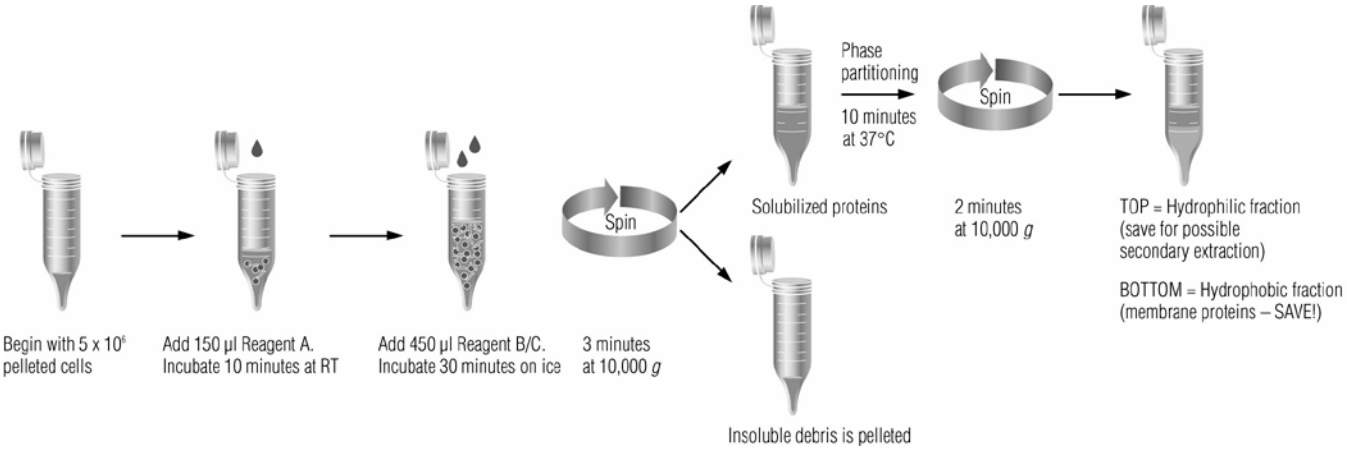

Figure 1. Summary of membrane protein extraction from mammalian cells.

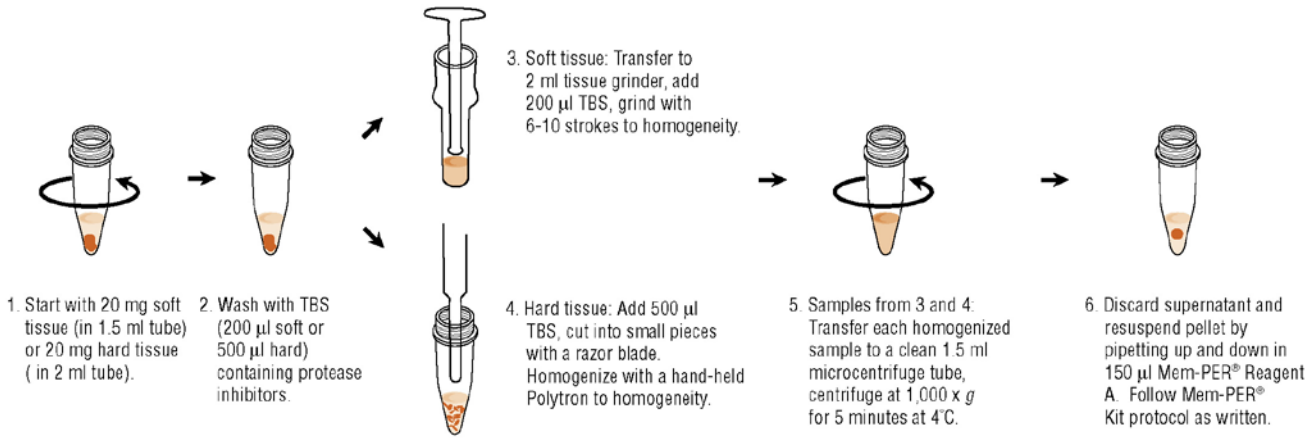

Figure 2. Summary of membrane protein extraction from soft and hard tissues.

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
23225	**Pierce BCA Protein Assay Kit**
89888	**Pierce SDS-PAGE Sample Prep Kit**
87780	**Pierce Detergent Removal Resin,** 10mL
78840	**Subcellular Protein Fractionation Kit**
34095	**SuperSignal™ West Femto Maximum Sensitivity Substrate**, 100mL
34075	**SuperSignal West Dura Extended Duration Chemiluminescent Substrate**, 100mL
34080	**SuperSignal West Pico Chemiluminescent Substrate**, 500mL

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