INSTRUCTIONS

PIERCE
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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 50-100 mammalian cell pellet fractions containing 5×10^6 cells each, 15 mg of wet yeast cell paste, or 20 mg of tissue

Kit Contents:

Mem-PER® Reagent A, 10 ml

Mem-PER® Reagent B, 25 ml

Mem-PER® Reagent C, 40 ml

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

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. First, cells are lysed with a detergent 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. 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.

Additional Material Required

- Protease inhibitors (e.g., Product No. 78415)
- For soft tissues a 2 ml Dounce Tissue Grinder, such as Kontes or Wheaton Tenbroeck, is required.
- For hard tissues a hand-held homogenizer for 0.5 to 1.5 ml samples, such as Brinkmann Polytron PT-1200CL, is required.
- Tris Buffered Saline (TBS; 0.025 M Tris, 0.15 M 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.

Protocol 1: Mammalian Cells

1. Isolate 5 x 10^6 cells per sample by centrifuging harvested cell suspensions at 850 × *g* for 2 minutes. Pellet cells (washed in PBS) in 1.7 ml microcentrifuge tubes.
2. Carefully remove and discard the supernatant.
3. Add 150 µl 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 5 µl of cell lysate onto a glass slide, add coverslip and view under a light microscope. Compare with 5 µl of the same number of intact cells in 150 µl 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 450 µl (e.g., for 10 extractions, combine 3.33 ml of Reagent C with 1.67 ml of Reagent B). Keep Reagents B and C at 4°C or on ice at all times.
6. Add 450 µl 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

1. Prepare approximately 150 mg of 405-600 µm 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, 15 mg of wet cell paste per assay.
3. Pellet cells by pulse centrifugation and carefully remove the supernatant.
4. Resuspend cells in 80 µl 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 720 µl (e.g., for 10 extractions, combine 4.8 ml of Reagent C with 2.4 ml of Reagent B). Keep Reagents B and C at 4°C or on ice at all times.
7. Add 720 µl 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

1. Place 20 mg of soft tissue in a 1.5 ml microcentrifuge tube. Add 200 µl of TBS to tissue, vortex briefly and discard wash.
2. Transfer rinsed tissue to a 2 ml tissue grinder. Add 200 µl TBS to tissue, and homogenize until an even suspension is obtained (approximately 6 to 10 strokes).
3. Transfer homogenate to a new 1.5 ml tube and centrifuge at 1,000 × *g* for 5 minutes at 4°C.
4. Discard supernatant and resuspend the pellet in 150 µl 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 450 µl (e.g., for 10 extractions, combine 3.33 ml of Reagent C with 1.67 ml of Reagent B). Keep Reagents B and C at 4°C or on ice at all times.
7. Add 450 µl 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

1. Place 20 mg of hard tissue in a 2.0 ml microcentrifuge tube. Add 500 µl TBS to tissue, vortex briefly and discard wash.
2. Add 500 µl TBS to 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 1.5 ml tube and centrifuge at 1,000 × *g* for 5 minutes at 4°C.
4. Discard the supernatant and resuspend the pellet in 150 µl 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 450 µl (e.g., for 10 extractions, combine 3.33 ml of Reagent C with 1.67 ml of Reagent B). Keep Reagents B and C at 4°C or on ice at all times.
7. Add 450 µl 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 Polyacrylamide Gel Electrophoresis

To determine extraction efficiency, perform SDS-PAGE and identify protein(s) of interest via a Western blot. To do so, normalize each set of fractions (one set includes debris and hydrophobic fractions) to the volume of the hydrophilic fraction. Dilute all fractions 2- to 5-fold to prevent band and lane distortion with the membrane fraction. Reagent B, diluted 4-fold with ultrapure water, can be used for normalization/dilution. Alternatively, use the PAGEprep™ Advance Kit (Product No. 89888) to remove detergent from the samples, which is recommended if the protein of interest is not abundant and a large volume of the membrane fraction is required for adequate detection. The PAGEprep™ Kit treatment is also recommended for samples prepared using two extractions, because the second extraction concentrates detergent, as well as for any proteins that migrate at or near the dye front, because detergent also migrates at this location and causes severe band distortion.

Preparation of Samples for Downstream Applications

The detergent in the membrane fraction must be reduced through dialysis for the sample to be compatible with many downstream applications, including protein assays such as the Micro BCA™ Protein Assay (Product No. 23235). Dialyze overnight at 4ºC against a buffer that contains 0.5% detergent (e.g., CHAPS or OTG) to maintain protein solubility as buffer exchange occurs. Slide-A-Lyzer® Dialysis Cassettes are suitable for samples greater than 100 µl and Slide-A-Lyzer® MINI Dialysis Units for samples between 10-100 µl. Dialyze against a volume that is 150-fold greater than the volume of the sample being dialyzed and change the dialysis buffer twice. When preparing dilutions of the dialyzed material for the Micro BCA™ Protein Assay, dilute the dialyzed membrane protein sample with a buffer containing 0.5% detergent to prevent protein precipitation.

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).

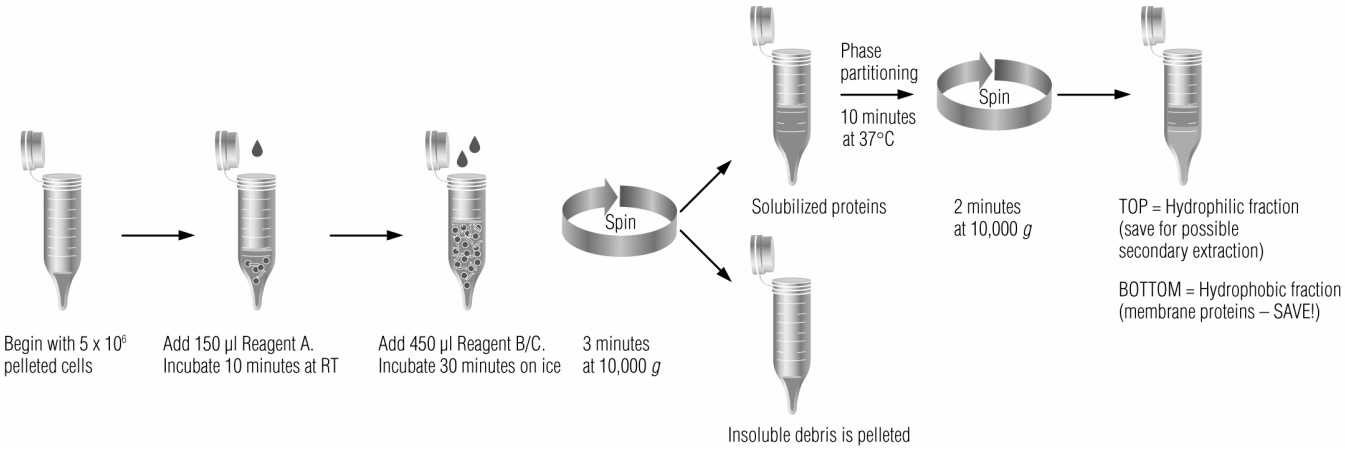

Figure 1. Summary of membrane protein extraction from mammalian cells.

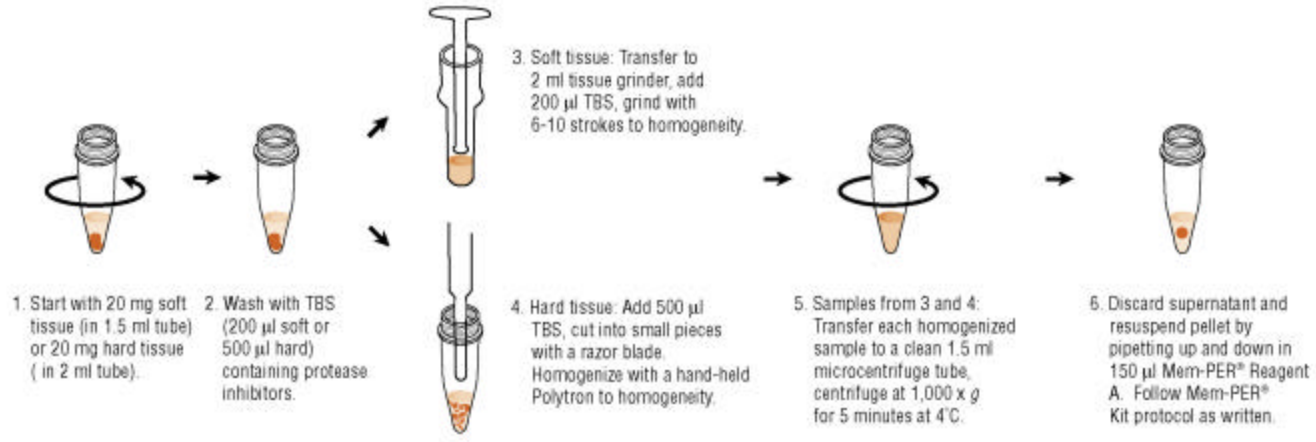

Figure 2. Summary of membrane protein extraction from soft and hard tissues.

Related Pierce Products

78410	**Halt™ Protease Inhibitor Cocktail Kit**
78415	**Halt™ Protease Inhibitor Cocktail Kit, EDTA-Free**
78420	**Halt™ Phosphatase Inhibitor Cocktail** (100X), 1 ml
23235	**Micro BCA™ Protein Assay Reagent Kit**
69570	**Slide-A-Lyzer® MINI Dialysis Units**, 10-100 µl capacity, 10K MWCO, 50 units
66383	**Slide-A-Lyzer® Dialysis Cassette Kit,** 0.1-0.5 ml, 10K MWCO, 10 each, contains cassettes, buoys, needles and syringes
89888	**PAGEprep™ Advance Kit**
34095	**SuperSignal® West Femto Maximum Sensitivity Substrate**, 100 ml
34075	**SuperSignal® West Dura Extended Duration Chemiluminescent Substrate**, 100 ml
34080	**SuperSignal® West Pico Chemiluminescent Substrate**, 500 ml
89864	**2-D Sample Prep for Membrane Proteins**
25200-25244	**Precise™ Protein Gels,** see catalog or web site for a complete listing

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