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INSTRUCTIONS

ProFoldin Membrane Protein Extraction Solutions

CATALOG NUMBERS

Membrane Protein Extraction Kit: MPE01-12S (Solutions #1 to #12)

Membrane Protein Extraction Kit Plus: MPE01-12P (Solutions #1 to #12)

Membrane Protein Extraction Solutions:

MPE01 (Solution #1) MPE02 (Solution #2) MPE03 (Solution #3) MPE04 (Solution #4) MPE05 (Solution #5) MPE06 (Solution #6)
MPE07 (Solution #7) MPE08 (Solution #8) MPE09 (Solution #9) MPE10 (Solution #10) MPE11 (Solution #11) MPE12 (Solution #12)

INTRODUCTION

ProFoldin's Membrane Protein Extract Solutions provide optimal conditions for efficient extraction and stabilization of membrane proteins from the cell membranes by using a variety of non-denaturing detergent and synthetic lipid analogs including alkyl saccharides (MPE01, MPE02), acyl-N-methylglucamide (MPE 04), bile acid salt (MPE 06), alkylaminoxide (MPE 07), alkylpolyethylenes (MPE 03, MPE 05, MPE 11), zwitterionic detergents (MPE 08, MPE 09, MPE 12), and synthetic phosphocholine derivative (MPE 10). The Membrane Protein Extraction Kit (MPE01-12S) includes 0.5 ml of 12 solutions for screening and micro-scale preparative extraction. The Membrane Protein Extraction Kit Plus (MPE01-12P) includes 5 ml of 12 solutions for screening and middle-scale preparative membrane protein extraction (10 – 15 mg of total membrane protein). The Membrane Protein Extraction Solution (MPE01 to MPE12) provides 40 ml of a specific solution for large-scale preparative extraction (80 - 120 mg of total membrane protein).

MEMBRANE PROTEIN EXTRACTION PROCEDURE

1. Membrane protein extraction test

Use the Membrane Protein Extraction Kit (MPE01-12S) or the Membrane Protein Extraction Kit Plus (MPE01-12P) to optimize the extraction conditions. Each test uses an amount of cell membranes with about 3 mg of total membrane proteins.

- (1) **Prepare cell membranes:** Different cell membranes are prepared using different protocols. In general, the cell membranes are isolated from the cell lysate by high-speed centrifugation, washed with a proper buffer and stored at -80°C. Transfer the membranes with about 0.2 – 0.3 mg of membrane proteins into 12 different microcentrifuge tubes^(a). Spin down the membranes at 100,000 x g for 45 min. Carefully remove the supernatant.
- (2) **Solubilize membrane proteins:** Thaw the 12 extraction solutions provided in the Kit^(a). Add 100 µl of each solution into different microcentrifuge tubes with the membrane pellet. Add proper protease inhibitors in the solutions. Make sure that the membranes are well suspended into the solutions by vortex and incubate the suspension at a proper temperature for 2 hr or overnight depended on the protein stability. Solubilization at different temperatures (0°C, room temperature or 37°C) may be tested. At the end of incubation, centrifuge the solubilization mixture and transfer each supernatant into a fresh tube for analysis.
- (3) **Analyze the solubilized membrane proteins:** Analyze the solubilized membrane proteins by SDS-PAGE and activity assays. Identify the optimal extraction solution for preparative membrane protein extraction and purification.

2. Preparative membrane protein extraction and purification

Use the optimal solubilization condition for preparative membrane protein extraction by increasing the amount of the extraction solution (Solution # 01 to 12) and cell membranes proportionally. For membrane protein purification, a mild detergent such as dodecyl maltoside with a 2-fold CMC concentration may be used in the purification buffer.

^(a) Warm the solution to solubilize any precipitates. Microcentrifuge tubes from Beckman (Cat# 357448) can be used.