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INSTRUCTIONS

ProFoldin
Spin-column Membrane Protein Folding Kits

Spin-column Membrane Protein Folding Screen Kit — Cat. No. MFC01-20
Small-scale Preparative Membrane Protein Folding Column Set #1 to #20 — Cat. No. MFC01S to MFC20S

INTRODUCTION

The Spin-column Membrane Protein Folding Kits are designed for folding membrane proteins from inclusion bodies using spin columns. The Spin-column Membrane Protein Folding Screen Kit (Cat. No. MFC01-20) includes 20 protein folding spin columns representing 20 different conditions with various detergents and lipids that form micelles or bicelles. The micellar or bicellar environments facilitate folding receptors, ion channels and other membrane proteins. The folded protein samples from the screen kit are used for SDS-PAGE and activity tests. Based on the test results, the optimal condition (the column number) is selected for preparative folding. For scale-up folding of membrane proteins, please use the Large-scale Preparative Membrane Protein Folding Column Set (Cat. No. MFC01 to MFC20).

Each **Spin-column Membrane Protein Folding Screen Kit (Cat. No. MFC01-20)** includes 160 µl of Reagent A, 160 µl of Reagent B, 1.1 m l of Reagent C and 20 prepacked spin-columns for 20 different protein folding conditions.

Each **Small-scale Preparative Membrane Protein Folding Column Set #1 to #20 (Cat. No. MFC01S to MFC20S)** includes 160 µl of Reagent A for columns #1 to 10 or Reagent B for columns #11 to 20, 1.1 m l of Reagent C and 10 identical prepacked spin-columns for the selected folding condition among the 20 conditions in the Screen Kit (Cat. No. MFC01-20).

PROTEIN FOLDING PROCEDURE

Please see the protocol of inclusion body preparation. The protein concentration is about 2 – 5 mg/ml.

(1) **Sample preparation:** Prepare Loading Sample A for columns #1 to 10 and Loading Sample B for columns #11 to 20. To prepare Loading Sample A, mix 130 µl of the solubilized inclusion bodies with 130 µl of Reagent A. To make Loading Sample B, mix 130 µl of the solubilized inclusion bodies with 130 µl of Reagent B.

(2) **Column preparation:** Spin the columns at 3200 rpm for 30 sec using a bench-top microcentrifuge. Remove the column bottom tips and caps. Place the columns into 1.5 ml-microcentrifuge tubes. Spin the columns at 1400 rpm for 2 min, and then transfer each column into a clean labeled 1.5-ml microcentrifuge tube.

(3) **Protein folding:** Load 25 µl of Loading Sample A onto each column from #1 to 10; Load 25 µl of Loading Sample B onto each column from #11 to 20. Spin the columns at 3200 rpm for 4 min. Discard the columns and incubate the eluent at 4ºC for 2 hr. Then add 50 µl of Reagent C into each eluent and incubate the solutions at 4ºC overnight. Spin the solutions at 14,000 rpm for 5 min and collect the supernatant for analysis.

ANALYSIS OF THE FOLDING PRODUCT

SDS-PAGE: Use SDS-PAGE to check protein solubility under each folding condition. To make the SDS-PAGE samples, mix 10 µl of the folding product from each column with 10 µl of water and 7 µl of 4 x SDS-PAGE loading buffer.

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Activity test: Use an activity assay (catalytic or binding activity) to check the protein activity. Make 10 to 20 fold dilution of the folding product in the assay. For example, if the assay reaction volume is 100 µl, add 5 µl to 10 µl of the folding product for each reaction. The assay buffer may include 1 mM dodecyl maltoside as the detergent.

Note: If there is no activity assay available, chromatographic behaviors can be used as an indicator of the protein folding state. A well folded membrane protein can be purified in buffers with a proper detergent (for example, 1 mM dodecyl maltoside). CD or SEC-MALS analysis is for purified protein samples only.

PROTEIN INCUSION BODY PREPARATION AND SOLUBILIZATION

Inclusion body isolation:

1. Resuspend the cell pellet in 20 ml of cell lysis buffer (20 mM Tris-HCl, pH 8, 100 mM NaCl, 2 mM DTT, 2 mM EDTA) for each liter of culture. Increase the volume proportionally for cell pellets from more than 1 L of culture.
2. Break the cells by passing the cell suspension French Press twice.
3. Centrifuge the broken cell suspension at 20,000 rpm for 20 min.
4. The crude inclusion bodies are in the pellet.

Inclusion body purification:

1. Resuspend the crude inclusion bodies in the cell lysis buffer plus 1 % Triton-100 by stirring at 4°C for 1 to 2 hours.
2. Centrifuge the suspension at 20,000 rpm for 20 min. Discard the supernatant.
3. Resuspend again the pellet in the cell lysis buffer without Triton.
4. Centrifuge the suspension at 20,000 rpm for 20 min. Discard the supernatant. The pellet is the purified inclusion bodies.

Inclusion body solubilization:

1. Estimate the amount of protein in the purified inclusion bodies. Add the volume of the solubilization buffer (20 mM Tris-HCl, pH 8.0, 8 M urea, 10 mM DTT) to make about 10 mg/ml protein concentration. The solubilization is performed by stirring the inclusion bodies with the solubilization buffer at room temperature for 2 to 4 hours. Most of the pellet should be solubilized.
2. Centrifuge the solubilization material at 30,000 rpm for 45 min. Save the supernatant as the solubilized inclusion bodies.

RELATED PRODUCTS

Membrane proteins:

Large-scale Preparative Membrane Protein Folding Column Set	Cat. No. MFC01 to MFC20
Dilution Membrane Protein Folding Screen Kit	Cat. No. MPS10-20

Soluble proteins:

Spin-column protein folding screen kit	Cat. No. SFC01-10
96-well protein folding plate	Cat. No. PFS096

For more information of protein folding or membrane protein extraction, please visit www.profoldin.com.