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INSTRUCTIONS

ProFoldin Spin-column Membrane Protein Folding Screen Kit

CATALOG NUMBER **MFC01-20** (Columns # 1 to #20)

INTRODUCTION

ProFoldin Spin-column Membrane Protein Folding Kit is specifically designed for folding membrane proteins from urea-solubilized inclusion bodies or other protein aggregates. The kit is composed of 20 protein folding spin columns and Reagents A, B and C. The 20 spin-columns represent 20 different conditions with various detergents and lipids that form micelles and bicelles. The micellar and bicellar environments facilitate folding receptors, ion channels and other membrane proteins. When the active protein is identified, preparative columns with the specific condition (the column number) are available for large-scale preparations of the folded membrane proteins.

PROTEIN FOLDING PROCEDURE

- (1) **Inclusion body solubilization:** Solubilize the inclusion bodies in 20 mM Tris-HCl, pH 7.0, 8 M urea, 10 mM DTT, 2 mM EDTA by stirring at room temperature for 4 hr. Centrifuge the solubilization material at 125,000xg for 30 min to remove any insoluble materials. Adjust the protein concentration to about 2 to 5 mg/ml.
- (2) **Sample preparation:** Prepare Loading Samples A and B. 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.
- (3) **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.
- (4) **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 4°C for 2 hr. Then add 50 µl of Reagent C into each eluent and incubate the solutions at 4°C 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.

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.

FOR PROTEINS WITH UNKNOWN FUNCTION OR NO ASSAY AVAILABLE

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. Preparative folding is needed for purification of the folded protein in milligram scales.