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INSTRUCTIONS

ProFoldin
Dilution Membrane Protein Folding Kits

CATALOG NUMBERS

Dilution Membrane Protein Folding Screen Kit: **MPS01-20** (Conditions #1 to #20)

Dilution Preparative Membrane Protein Folding Kits:

MPP01 (condition #1)	**MPP02** (condition #2)	**MPP03** (condition #3)	**MPP04** (condition #4)	**MPP05** (condition #5)
MPP06 (condition #6)	**MPP07** (condition #7)	**MPP08** (condition # 8)	**MPP09** (condition #9)	**MPP10** (condition #10)
MPP11 (condition #11)	**MPP12** (condition #12)	**MPP13** (condition #13)	**MPP14** (condition #14)	**MPP15** (condition #15)
MPP16 (condition #16)	**MPP17** (condition #17)	**MPP18** (condition #18)	**MPP19** (condition #19)	**MPP20** (condition #20)

INTRODUCTION

ProFoldin Dilution Membrane Protein Folding Screen Kit (catalog # MPS01-20) provides 20 optimized conditions for screens of membrane protein folding conditions. About 70 µg of urea-solubilized proteins from inclusion bodies are used for each condition. Once the folding conditions are identified, preparative folding kits (see catalog numbers above) are available for preparative scale folding. Each preparative folding kit is for folding 5 mg of urea-solubilized proteins. The condition number is identical to the Solution S number in the kit.

The Dilution Membrane Protein Folding Screen Kit (catalog # MPS01-20) includes 0.4 ml of Reagent A, 0.4 ml of Reagent B, 7.2 ml of Reagent C, 25 ml of 20 Solution S.

PROTEIN FOLDING PROCEDURE

Folding Screen

(1) Solubilize the inclusion bodies in 20 mM Tris-HCl, pH 7.0, 8 M urea, 10 mM DTT, 5 mM EDTA at room temperature for 4 hr. Centrifuge the solubilization material at 125,000 x g for 30 min to remove any insoluble materials. If the protein purity is < 50 %, purification of the denatured protein is recommended. Adjust the protein concentration to about 5 to 10 mg/ml.

(2) For Conditions 1 to 10: Pre-incubate Solution S1 to S10 at 4ºC. Mix 105 µl of the urea-solubilized protein with 55 µl of Reagent A to make Sample A. Incubate Sample A at room temperature for 2 hr. Then mix 15 µl of Sample A with each Solution S1 to S10 (350 µl) at 4ºC for 2 hr.
For Conditions 11 to 20: Warm Reagent B to room temperature to solubilize any precipitates. Pre-incubate Solution S11 to S20 at room temperature. Mix 105 µl of the urea-solubilized protein with 55 µl of Reagent B to make Sample B. Incubate Sample B at room temperature for 2 hr. Then mix 15 µl of Sample B with each Solution S11 to S20 (350 µl) at room temperature for 2 hr.

(3) Add 100 µl of Reagent C into each folding solution and incubate all solutions at 4ºC overnight.

(4) Spin the solutions at 14,000 rpm for 10 min and collect the supernatant for analysis of the folded protein.

Preparative folding

Use the optimized condition to scale-up the folding reaction. Follow the same procedure as that in the screen experiment but in a larger volume (70 fold) of solutions: 700 µl of urea-solubilized protein; 350 µl of Regent A (for

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condition # 1 to 10) or Reagent B (for condition # 11 to 20); 24.5 ml of Solution S; 7 ml of Reagent C. Centrifuge the final folding solution at 50,000 x g for 20 min and collect the supernatant. Dialyze the supernatant against the column buffer to remove the extra salt (0.5 M) or EDTA (1 mM) if ion-exchange or Ni column is used for purification of the folded protein.

Protein inclusion body preparation and solubilization

The following protocols of protein inclusion body preparation and solubilization are recommended.

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 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.

Protein folding kits using solubilized inclusion bodies:

Soluble proteins:

Spin-column protein folding screen kit	Catalog number: SFC01-10
96-well protein folding plate	Catalog number: PFS096

Membrane proteins:

Dilution Membrane Protein Folding Screen Kit	Catalog number: MPS10-20
Spin-column Membrane Protein Folding Screen Kit	Catalog number: MFC01-20

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