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INSTRUCTIONS

ProFoldin
Dilution Membrane Protein Folding Kits

CATALOGUE NUMBER: P4-1084

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.

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) Warm Reagent A and Reagent B to room temperature to solubilize any precipitates. Pre-incubate Solution S1 to S10 at 4ºC and Solution S11 to S20 at room temperature. Mix 105 µl of the urea-solubilized protein with 55 µl of Reagent A to make Sample A. Mix 105 µl of the urea-solubilized protein with 55 µl of Reagent B to make Sample B. Incubate Sample A and Sample B separately at room temperature for 2 hr.

(3) Mix 15 µl of Sample A with each Solution S1 to S10 (350 µl) at 4ºC for 2 hr. Mix 15 µl of Sample B with each Solution S11 to S20 (350 µl) at room temperature for 2 hr.

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

(5) 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 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.