



INSTRUCTIONS

ProFoldin Preparative Membrane Protein Folding Column Sets

CATALOGUE NUMBERS: P4-1044 (MFC01) to P4-1082 (MFC20)

MFC01 (column #1)	MFC02 (column #2)	MFC03 (column #3)	MFC04 (column #4)
MFC05 (column #5)	MFC06 (column #6)	MFC07 (column #7)	MFC08 (column #8)
MFC09 (column #9)	MFC10 (column #10)	MFC11 (column #11)	MFC12 (column #12)
MFC013 (column #13)	MFC14 (column #14)	MFC15 (column #15)	MFC16 (column #16)
MFC17 (column #17)	MFC18 (column #18)	MFC019 (column #19)	MFC20 (column #20)

Reagent A or Reagent B, Reagent C and Solution S which are used in the protein folding procedure (see below) are shipped with each kit. Reagent A is for column #1 to #10; Reagent B is for column #11-20.

INTRODUCTION

The Preparative Membrane Protein Folding Column Sets are used for preparative membrane protein folding after the folding condition has been identified by the Spin-column Membrane Protein Folding Screen Kit (Catalog # MFC01-20). The column number represents the specific folding condition. Each Column Set includes 4 identical preparative protein folding columns and reagents for folding about 5 mg of urea -solubilized inclusion body proteins.

PROTEIN FOLDING PROCEDURE

- 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 125,000 x g for 30 min to remove any insoluble materials. Adjust the protein concentration to about 2 – 4 mg /ml.
- Sample preparation:** To make the loading sample, mix 5 ml of the solubilized inclusion bodies with 5 ml of Reagent A or B (Reagent A for column #1 -10, Reagent B for column #11-20). Incubate the loading sample at room temperature for 2 hr.
- Protein folding:**
 - Step 1:** Cut off the bottom tip of each column and let the buffer run through the column, load 2.5 ml of the loading sample. Let the sample run completely into the column. Provide a little pressure on the top of the column with the column cap if the flow stops. Discard the flow-through.
 - Step 2:** Elute the protein with 3.5 ml of Solution S. The Solution S number matches the column number. Collect and incubate the eluent at 4°C for 2 hr. Discard the columns.
 - Step 3:** Mix 8 ml of Reagent C with the 14 ml of eluent from the 4 columns and incubate the solution at 4°C for 2 hr to overnight. Remove the precipitate (if there is any) by centrifugation.

PROTEIN PURIFICATION AFTER FOLDING

The folded protein can be purified by affinity, ion-exchange or gel filtration column chromatography. Some proteins are sensitive to low salt. Concentration by ultrafiltration may also cause concentration of the detergent that may denature the folded membrane protein. To be cautious, brief dialysis of the protein solution against a buffer with a moderate salt concentration at 4°C is recommended. Following is a Q-Sepharose column purification protocol:

- Dialyze the protein solution against 40 volumes of 20 mM Tris-HCl, pH 8.5, 125 mM NaCl, 2 mM DTT, 1 mM dodecyl maltoside, 2 mM EDTA buffer at 4°C for 1 to 2 hr. Remove any precipitates by centrifugation.
 - Equilibrate a Q-Sepharose column with a low salt buffer (the same as the dialysis buffer). Load the dialyzed protein solution. Wash the loaded column with 10 column volumes of the same buffer. Elute the protein with a salt gradient from 125 mM to 1 M.
 - Any further protein purification step may follow as purification of native membrane proteins.
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