



ProFoldin

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INSTRUCTIONS

ProFoldin

Large-scale Preparative Protein Folding Column Sets

CATALOG NUMBERS

PFC01 (column # 1)

PFC02 (column # 2)

PFC03 (column # 3)

PFC04 (column # 4)

PFC05 (column # 5)

PFC06 (column # 6)

PFC07 (column # 7)

PFC08 (column # 8)

PFC09 (column # 9)

PFC10 (column # 10)

INTRODUCTION

The Large-scale Preparative Protein Folding Column Sets are used for preparative protein folding after the folding condition has been identified by the Spin-column Protein Folding Screen Kit (Catalog number SFC01-10). The column number represents the specific folding condition. Each Large-scale Preparative Protein folding Column Set includes 4 identical preparative protein folding columns and reagents for folding 10 to 20 mg of guanidine hydrochloride or urea-solubilized inclusion body proteins.

The **Large-scale Preparative Protein Folding Column Sets** (Catalog No. PFC01 to PFC10) includes 4 columns, 5.4 ml of Solution A, 15.4 ml of Solution C and 8.4 ml Solution B.

PROTEIN FOLDING PROCEDURE

Inclusion body preparation and solubilization

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.
2. Break the cells by passing the cell suspension French Press twice and centrifuge the broken cell suspension at 20,000 rpm for 20 min.
3. Resuspend the pellet in the cell lysis buffer plus 1 % Triton-100 by stirring at 4°C for 1 to 2 hours and centrifuge the suspension at 20,000 rpm for 20 min. Discard the supernatant.
4. Wash the pellet in the cell lysis buffer without Triton by suspension and centrifugation.
5. Solubilize the inclusion bodies by stirring the pellet in 20 mM Tris-HCl, pH 8.0, 6 M guanidine hydrochloride (or 8 M urea), 10 mM DTT at room temperature for 2 hours. Then centrifuge the solubilization material at 30,000 rpm for 45 min. Save the supernatant as the solubilized inclusion bodies.

Protein folding using the large-scale preparative protein folding columns

The columns and reagents are cooled to 2 - 8°C. The experiment is performed in a cold room. An easy way to set up the columns is to use a test tube rack to hold the columns and place a tip box cover under the rack to receive the solution from the columns.



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1. Sample preparation

To make the loading sample, mix 5 ml of the solubilized inclusion bodies with 5 ml of Solution A. Incubate the mixture (the loading sample) for 5 min.

2. Column preparation

Cut off the column bottom pointing tips and let the buffer run through the columns.

3. Protein folding

- (1) load 2.5 ml of the loading sample per column. Let the sample completely run into the columns.
- (2) Elute the protein with 3.5 ml of Solution C per column. The solution C number matches the column number. Collect and incubate the eluent at 4°C for 2 to 4 hr. Discard the columns.
- (3) Mix 8 ml of Solution B^(a) 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.

Note: If solution B forms precipitate during storage, warm it to room temperature to solubilize the precipitate, then cool it back to 4°C before use.

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. 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:

- (1) Dialyze the protein solution against 40 volumes of 20 mM Tris-HCl, pH 8.5, 50 mM NaCl, 2 mM DTT, 2 mM EDTA buffer at 4°C for 2 to 4 hr. Remove any precipitates by centrifugation.
- (2) 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.
- (3) Any further protein purification step may follow as purification of regular soluble native proteins.

RELATED PRODUCTS

Spin-column Protein Folding Screen Kit

Catalog number: SFC01-10

96-well protein folding plate

Catalog number: PFS096

Spin-column Membrane Protein Folding Screen Kit

Catalog number: MFC01-20