



INSTRUCTIONS

ProFoldin Spin-Column Protein Folding Screen Kit

CATALOGUE NUMBER: P4-1000 (SFC01-10)

INTRODUCTION

ProFoldin protein folding columns are designed to produce active proteins from guanidine hydrochloride or urea-solubilized inclusion bodies or protein aggregates formed during protein purification or storage. The Spin-column Protein Folding Screen Kit includes 10 spin-columns that represent 10 optimized and diversified protein folding conditions. The folded protein samples from the screen kit are used for SDS-PAGE and activity tests. Based on the test results, the optimal condition (the column number) is selected for preparative folding. The Large-scale Preparative Protein Folding Column Set is for folding of 10 to 20 mg of denatured proteins.

PROTEIN FOLDING PROCEDURE

Perform the following experiment in a 4°C cold room.

- (1) Solubilize inclusion bodies ^(a). The protein concentration is about 5 – 10 mg/ml.
- (2) Spin the columns at 3200 rpm for 1 min using a bench-top microcentrifuge to set down the resin. Remove the column bottom tips and caps. Place the columns into 1.5 ml-microcentrifuge tubes and spin the columns at 1400 rpm for 2 min. Transfer each column into a clean labeled 1.5-ml microcentrifuge tube.
- (3) To make the loading sample, mix 130 µl of the solubilized inclusion bodies with 130 µl of Solution A. Incubate the mixture (the loading sample) for 5 min.
- (4) Load 25 µl of the loading sample onto each column and spin the columns at 3200 rpm for 4 min. Discard the columns and incubate the eluent at 4°C for 2 to 4 hr.
- (5) Mix 50 µl of Solution B ^(b) with the eluent from each column and incubate the solution at 4°C for 2 hr to overnight. Spin the solution at 14,000 rpm for 5 min and collect the supernatant (the folding product) 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.

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 protein can be purified as a normal soluble protein. CD or SEC-MALS analysis is for purified protein samples only. Preparative folding is needed for purification of the folded protein in milligram scales.

^(a) The inclusion bodies are solubilized in 20 mM Tris-HCl, pH 7.0, 7 M guanidine hydrochloride, 10 mM DTT, 2 mM EDTA by constant stirring at room temperature for 2 hr to overnight. The solubilization material is centrifuged at 125,000 x g for 30 min to remove any insoluble materials.

^(b) 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.