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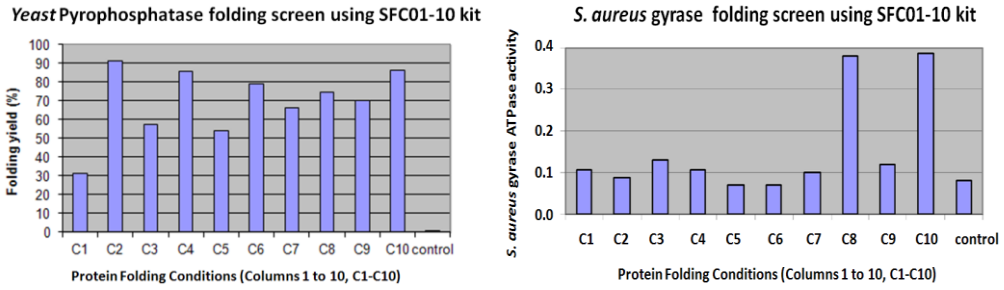
INSTRUCTIONS

ProFoldin
Spin-column Protein Folding Screen Kit

CATALOG NUMBER 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 expression, purification or storage. Different proteins are folded under different conditions. The Spin-column Protein Folding Screen Kit includes 10 spin-columns that represent 10 optimized and diversified protein folding conditions including conditions allowing disulfide bond formation and reducing 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 (PFC01 to PFC10) is for folding of 10 to 20 mg of denatured proteins.



The **Spin-column Protein Folding Screen Kit** (Catalog No. SFC01-10) includes 10 spin columns, 160 µl of Solution A and 600 µl of 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

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solubilization material at 30,000 rpm for 45 min. Save the supernatant as the solubilized inclusion bodies.

Protein folding screen using the spin column kit

The columns and reagents are cooled to 2 - 8°C. The experiment is performed in a cold room.

1. Mix 130 µl of the solubilized inclusion bodies with 130 µl of Solution A. Incubate the mixture (the loading sample) for 5 min.
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. 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 4ºC for 2 to 4 hr.
4. Mix 50 µl of Solution B with the eluent from each column and incubate the solution at 4ºC for 2 hr to overnight. Spin the solution at 14,000 rpm for 5 min and collect the supernatant for analysis.

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

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.

References:

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