



## INSTRUCTIONS

# ProFoldin Protein Folding Spin-Columns

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**CATALOGUE NUMBERS: P4-1002 (SFC01) to P4-1020 (SFC10)**

**Spin-Column Protein Folding Screen Kit:**

**SFC01-10** (Columns # 1 to #10)

**Small-Scale Preparative Protein folding Column Sets:**

<b>SFC01</b> (column # 1)	<b>SFC02</b> (column # 2)	<b>SFC03</b> (column # 3)	<b>SFC04</b> (column # 4)
<b>SFC05</b> (column # 5)	<b>SFC06</b> (column # 6)	<b>SFC07</b> (column # 7)	<b>SFC08</b> (column # 8)
<b>SFC09</b> (column # 9)	<b>SFC10</b> (column # 10)		

Solution A and Solution B which are used in the protein folding procedure (see below) are shipped with each kit.

## INTRODUCTION

ProFoldin Protein Folding Columns are effective and easy-to-use tools for protein folding screens and preparative protein folding. The columns are designed to produce active proteins from urea- or guanidine hydrochloride (GdnHCl)-solubilized inclusion bodies or other protein aggregates. The 10 columns in the Spin-column Protein Folding Screen Kit (Catalog # SFC01-10) represent 10 optimized and diversified protein folding conditions. The 10 columns in each Small-scale Preparative Protein folding Column Set are identical to one column in the Screen kit. For example, the column set with catalog number SFC05 contains 10 columns with column # 5. The column # in the Small-scale Preparative Protein folding Column Set matches the column # in the Spin-column Protein Folding Screen Kit. Please visit our website ([www.profoldin.com](http://www.profoldin.com)) for more information.

## PROTEIN FOLDING PROCEDURE

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

- (1) Prepare a solution of solubilized inclusion bodies<sup>(a)</sup> with a protein concentration of 5 - 10 mg/ml.
- (2) Pre-spin the columns at 3200 rpm for 1 min using a standard bench-top microcentrifuge. Remove the bottom tips and caps of the columns. Place the columns into 1.5 ml-microcentrifuge tubes. Spin the columns at 1400 rpm for 2 min, and then 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 4 hr.
- (5) Mix 50 µl of Solution B<sup>(b)</sup> with each eluent and incubate the solution at 4°C overnight. Spin the solution at 14,000 rpm for 5 min and collect the supernatant for analysis or purification of the folded protein.

### Notes:

<sup>(a)</sup> It is recommended that the inclusion bodies are solubilized by stirring the inclusion bodies with a buffer composed of 20 mM Tris-HCl, pH 7.0, 7 M GdnHCl (or 8 M urea), 10 mM DTT, 2 mM EDTA at room temperature for 4 hr. The solubilization material is centrifuged at 125,000 x g for 30 min to remove any insoluble materials.

<sup>(b)</sup> 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.

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