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INSTRUCTIONS

ProFoldin
Large-Scale Preparative Protein Folding Column Sets

CATALOGUE NUMBERS: P4-1022 (PFC01) to P4-1040 (PFC10)

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)

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

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 # 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.

PROTEIN FOLDING PROCEDURE

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. The columns are chilled at 4ºC if they are not yet.

(1) Solubilize inclusion bodies [(a)]. The protein concentration is about 2 – 4 mg/ml. Make the loading sample for the 4 columns by mixing 5 ml of the solubilized inclusion bodies with 5 ml of Solution A.
(2) Perform the following experiment in a 4ºC cold room. Cut off the column bottom tips and let the buffer run through the columns, load 2.5 ml of the loading sample per column. Let the sample completely run into the columns.
(3) 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 4ºC for 2 to 4 hr. Discard the columns.
(4) Mix 8 ml of Solution B[(b)] with the 14 ml of eluent from the 4 columns and incubate the solution at 4ºC 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. To be cautious, brief dialysis of the protein solution against a buffer with a moderate salt concentration at 4ºC 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, 125 mM NaCl, 2 mM DTT, 2 mM EDTA buffer at 4ºC for 1 to 2 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 from 125 mM to 1 M.
(3) Any further protein purification step may follow as purification of regular soluble native proteins.

Notes:

[(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 4ºC before use.