



**ProFoldin Protein Folding Services**  
290 Turnpike Road, Suite 6, Number 321  
Westborough, MA 01581-2843  
FAX: (508) 845-9258  
[www.profoldin.com](http://www.profoldin.com)  
[info@profoldin.com](mailto:info@profoldin.com)

## INSTRUCTIONS

# ProFoldin 96-Well Protein Folding Plate

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### Catalog number: PFS096

**Components:** One 96-well plates with 96 Protein Folding Solutions, 150  $\mu$ l each well; 1.5 ml of Inclusion Body Solubilizer; 1.5 ml of Neutralizer.

### INTRODUCTION

ProFoldin 96-Well Protein Folding Plate (catalog # PFS096) provides 96 diversified conditions for protein folding screens. The 96 folding conditions include various pHs, salt concentrations and additives. About 25  $\mu$ g of guanidine hydrochloride-solubilized proteins from inclusion bodies are used for each condition. Once the folding conditions are identified, preparative folding solutions for specific conditions (well positions) are available for preparative scale folding.

### PROTEIN FOLDING PROCEDURE

- (1) **Sample preparation:** Prepare a solution of solubilized inclusion bodies<sup>(a)</sup> with a protein concentration of 5 - 10 mg/ml.
- (2) **Plate preparation:** Thaw the Protein Folding Plate at room temperature. Spin the plate at 1000 x g for 1 min to collect all the solutions into the wells. Then pre-incubate the plate at 4°C for 30 min. Gently remove the plate cover before adding or taking solutions and put the cover back after adding or taking solutions at steps (3) through (5).
- (3) **Dilution:** Dilute 2.5  $\mu$ l of the above protein solution into the solution in each well. Then incubate the plate at 4°C for 4 hours.
- (4) **Neutralization:** Mix 8  $\mu$ l of Neutralizer<sup>(b)</sup> with the solution in each well. Incubate the plate at 4°C overnight.
- (5) **Analysis:** Spin the plate at 1000 x g for 10 min. Analyze the protein folding product in each well<sup>(c)</sup>.

### Notes:

<sup>(a)</sup> If the protein purity of the inclusion bodies is < 70 %, it is recommended to purify the protein by gel filtration or ion exchange chromatography under denaturing conditions. His-tagged proteins can be purified on a Ni-column. If the protein purity is > 70 %, the solubilized inclusion bodies can be directly used for folding. To do so, solubilize about 1.5 - 3 mg of the inclusion body protein in 0.3 ml of Inclusion Body Solubilizer (20 mM TrisHCl, pH 7.5, 6 M GdnHCl, 5 mM DTT, 1 mM EDTA) by stirring or vortexing or nutating at room temperature for 4 hr or overnight. Then centrifuge the solubilization material at 125,000 x g for 30 min to remove any insoluble materials.

<sup>(b)</sup> If Neutralizer forms precipitate during storage, warm it to room temperature to solubilize the precipitate, then cool it back to 4°C before use.

<sup>(c)</sup> The protein activity of the supernatant in each well is measured to determine the optimal folding conditions for preparative folding.