**ProFoldin**
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# INSTRUCTIONS

# ProFoldin
# 96-Well Protein Folding Plate

## Catalog number: PFS096

**Components**: One 96-well plate with 96 protein folding solutions, 500 µl in each well of the mother plate; 1.4 ml of Inclusion Body Solubilizer; 4 ml of Neutralizer and a clear 96-well plate.

### 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. Each experiment uses 100 µl of the solutions from the mother plate. Each mother plate contains 500 µl of solutions in each well and can be used for multiple experiments of folding various proteins. About 20 µg 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:** Please see the protocol of inclusion body preparation. The protein concentration is about 10 mg/ml.
2. **Plate preparation:** Spin the plate at 1000 x g for 1 min to collect all the solutions at the bottoms of the wells. Gently remove the plate cover and transfer 100 µl of the solutions to the clear plate. Pre-incubate the plate at 4ºC and keep the reagents at 4ºC for the entire protein folding process.
3. **Dilution:** Dilute 2 µl of the above protein solution into the solution in each well. Then incubate the plate at 4ºC for 4 hours.
4. **Neutralization**: Mix 8 µl of Neutralizer with the solution in each well. Incubate the plate at 4ºC overnight.

### ANALYSIS OF THE FOLDING PRODUCT

- **Solubility**

The unfolded protein forms precipitate that is visible and can detected by light scattering measurement at 405 nm ($OD_{405}$). The solubilized protein can be analyzed SDS-PAGE. To do so, spin the plate at 1000 x g for 10 min and mix 10 µl of the supernatant from each well 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.

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- **For proteins with unknown function or no assay available**

A well folded protein can be purified by ion-exchange, affinity or size-exclusion columns in native buffers. CD or SEC-MALS analysis is for purified protein samples only.

**Inclusion body isolation:**

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. Increase the volume proportionally for cell pellets from more than 1 L of culture.
2. Break the cells by passing the cell suspension French Press twice.
3. Centrifuge the broken cell suspension at 20,000 rpm for 20 min and save the pellet as the crude inclusion bodies.
4. Resuspend the crude inclusion bodies in the cell lysis buffer plus 1 % Triton-100 by stirring at 4°C for 1 to 2 hours.
5. Centrifuge the suspension at 20,000 rpm for 20 min. Discard the supernatant.
6. Resuspend again the pellet in the cell lysis buffer without Triton.
7. Centrifuge the suspension at 20,000 rpm for 20 min. Discard the supernatant. The pellet is the purified inclusion bodies.

**Inclusion body solubilization:**

Use 6 M urea or guanidine hydrochloride to solubilize the protein.

1. Estimate the amount of protein in the purified inclusion bodies by SDS-PAGE. Add the volume of the solubilization buffer (20 mM Tris-HCl, pH 8.0, 6 M guanidine hydrochloride or urea, 10 mM DTT) to make about 10 mg/ml protein concentration. The solubilization is performed by stirring the inclusion bodies with the solubilization buffer at room temperature for 2 to 4 hours. Most of the inclusion body protein should be solubilized.
2. Centrifuge the solubilization material at 30,000 rpm for 45 min. Save the supernatant as the solubilized inclusion bodies.

**Related Products**

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| Spin-column protein folding screen kit | Catalog number: SFC01-10 |
| Dilution Membrane Protein Folding Screen Kit | Catalog number: MPS10-20 |
| Spin-column Membrane Protein Folding Screen Kit | Catalog number: MFC01-20 |
| Membrane Protein Extraction Kit | Catalog number: MPE01-12S |
| Easy Protein Assay Reagent | Catalog number: EPA001 |
| Nucleic Acid Removal Kit | Catalog number: NAR911 |
| Protein Stability and Aggregation Assay Kit | Catalog number: PSA200 |

For more information of protein preparation, protein activity function assays and other biochemical assays, please visit www.profoldin.com or contact our technical support at info@profoldin.com.