Protein Foldase™

Protein Folding Optimization Kit

INTRODUCTION

Recombinant proteins are widely expressed in bacteria. When expressed in bacteria, recombinant proteins often form inclusion bodies (i.e., protein aggregates), especially when expression is at high level. Reasons for the formation of inclusion bodies is not understood, however, it is believed that inclusion bodies are partially or incorrectly folded proteins. Because of the aggregate nature of inclusion bodies, they are easily separated from bacterial cytoplasmic proteins by centrifugation, giving effective purification step. Once protein aggregates or inclusion bodies are formed, it is very difficult to solubilize them. The IBS™ Buffer (i.e. Inclusion Bodies Solubilization buffer) is designed to solubilize inclusion bodies.

The Protein *Foldase*™ kit is designed to simplify the optimization of protein folding procedures. The kit is based on a proprietary combination of denaturing agents for solubilization of protein in inclusion bodies and folding protocols based on rapid dilution of denatured protein into a proprietary folding mix (e.g., *Foldase*™). Given the fact that proteins have unique folding properties, the kit offers a selection of successfully used folding mixes and simple to follow protocols that allows users the option to optimize folding protocol. The folding mixes include, Foldase™-I, Foldase™-II, Foldase™-III, and Foldase™-IV. These folding mixes are based on successfully used folding agents Cyclodextrin, Polyethylene Glycol, ND SB-201, and oxidizing-reducing agents (proprietary agents), respectively. However, there is no guarantee that every protein can be successfully folded into biologically active protein. Nevertheless, use of this kit simplifies the optimization of folding protocol. After optimization of folding protocol, any *Foldase*™ reagent may be re-ordered in larger quantity for preparative scale works.

Compatibility

PROTEIN-*Foldase*™ and the agents included in this kit are compatible with many downstream applications. Protein *Foldase*™ and agents included are also compatible with NI™ protein assay and PAGE-Perfect™ kit for running SDS-PAGE analysis (see Related Products for more information).

The kit components are suitable for 12-80 tests. Depending on reagents concentration used up to 80 tests are possible. Additional items may be purchased separately. The Protein Foldase™ Kit consists of the following items:

ITEM(S) SUPPLIED	Cat # 786-185
[1X] Protein Foldase™-I	30ml
[2X] Protein Foldase™-II	30ml
[2X] Protein Foldase™-III	15ml
[20X] Protein Foldase™-IV	1.5ml
DTT [1M in 1ml]*	4 Vials
Reaction Cups	12 cups
IBS™-Buffer.	50ml
Foldase™ Dilution Buffer	50ml

* SEE PREPARATION BEFORE USE

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store Foldase™-IV at -20°C and rest of the items at 4°C.

Protein Folding

When recombinant proteins form inclusion bodies, they cannot be used until the protein in the inclusion bodies is solubilized in strong denaturing agents and then allowed to correctly fold into biologically active forms. The problem is largely how to remove the denaturing agent (or lower the concentration) to allow folding of protein while at the same time preventing aggregation. Because every protein has unique folding properties, it is very difficult to prepare a universal folding protocol. Therefore, optimal folding protocol for any given protein must be empirically determined. Developing folding protocol often requires a great deal of scientific literature research and experimentation.

Two schools of thought exist for folding denatured protein. Several published methods suggest a gradual removal of denaturing & solubilization agent, preferable by dialysis. This method often does not work because protein in solution is exposed to a decreasing concentration of denaturant over a few hours and when exposed to an intermediate concentration of denaturant, the protein is prone to aggregation. London et al. (Eur. J. Biochem; vol 37, pp 409-415) have shown that folding of tryptophanase would reach a minimum when folding mix was incubated at an intermediate denaturant concentration. However, given the simplicity of this method, dialysis method is worth trying. There is growing consensus that successful protein folding requires denaturation and solubilization of aggregated protein, followed by rapid dilution of protein, which lowers the concentration of the denaturant to a level where protein begins to fold. Several researchers have added agents such as detergents, reducing agents, polyethylene glycol, etc. to limit the aggregation during rapid folding steps.

Preparation Before Use

*Dissolve the supplied DTT in DI water, final volume 1ml/vial to give 1M concentration. Store at –20^{o}C.

Isolation of Inclusion Bodies

For inclusion bodies isolation use Bacterial-PE LB™ (Cat. #786-176). Briefly, incubate bacterial cells in Bacterial-PE LB™ until lysis is complete. At the end of incubation, centrifuge the lysate at 30,000xg for 30 minutes at 4^{o}C. Collect the pellet and wash twice with 1:10 fold diluted Bacterial- PE LB™. Collect the inclusion bodies for folding procedures.

Inclusion Body Solubilization:

1. Determine the wet weight of inclusion bodies. Suspend the inclusion bodies in an appropriate volume of IBS™-Buffer.
   For example, subtract the weight of the tube with the total weight of the tube plus inclusion bodies. For each 100 mg wet weight of inclusion bodies add 1ml IBS™ Buffer.

2. Incubate the inclusion bodies suspension for 1 hour at 4^{o}C.

3. Remove insoluble material by centrifugation at 100,000xg for 10 minutes. It is important to remove existing aggregates that may act as nuclei to trigger aggregation during folding procedure.

Protein Folding Protocol

The entire protocol should be performed in cold (at 4-8^{o}C) unless indicated otherwise.

1. Solubilize the inclusion bodies in IBS™-Buffer, as described above.
2. Determine the protein concentration of solubilized inclusion bodies (using Non-Interfering Protein assay, Cat. # 786-005). Adjust the protein concentration with IBS™ Buffer to approximately 1mg/ml.
3. Prepare a *Foldase*™ either by diluting with *Foldase*™ Dilution Buffer or mixing with other *Foldase*™. As a general guide, use 1X-*Foldase*™ (I, II, III or IV) first. Depending on results, two *Foldase*™ (I, II, III or IV) may be mixed together to a final concentration of 0.1 to 1X.

Foldase™-I	may be used as 0.25X to 1X
Foldase™-II	may be used as 0.1 X to 1X
Foldase™-III	may be used as 0.5 X to 1X
Foldase™-IV	may be used as 0.5X to 1X

4. Transfer 10 ml *Foldase*™-I (or -II, III, IV or a combination of two *Foldase*™) into a reaction cup. Add DTT (1M) to a final concentration of 25mM (25ul/ml).
   NOTE: *Do not add DTT in any solution containing Foldase*™*- IV. The Foldase*™*- IV is supplied as suspension, allow the suspension to thaw and vortex the vial before taking an aliquot out.*

   Place a small magnetic stirrer into the reaction cup and stir the solution vigorously.

5. Using a 1ml pipetor or a syringe, take 1ml solution of the inclusion bodies (solubilized and adjusted to 1mg/ml protein concentration) and rapidly introduce into the vigorously stirring Foldase™ solution.

6. Keep the Foldase™ solution mixing vigorously for another 30-40 seconds after the addition. Incubate the solution with gentle stirring for 2h at 4°C.

7. Remove any remaining IBS™-buffer by dialysis (with 1-4 kDa mol. wt. cut-off dialysis membrane) against the Bacterial PE LB™ or an appropriate buffer. Use 250ml of dialysis buffer and allow at least two changes of buffer.

8. Assay the protein solution for biological activity and success of folding. Measuring the solution turbidity at 400nm is a good indication of protein aggregation.

IMPORTANT NOTE: *The protocol suggested here are only for starting. For further optimization, the key parameters that need to be adjusted are protein concentration, the residual IBS™-Buffer concentration, length of incubation, and temperature.*

For Preparative & Large Scale Works:
After optimization of the folding protocol, any *Foldase™* reagent may be re-ordered in larger quantity (100ml each) for preparative scale works.
Instructions for Use - Increase the volumes proportionately and use the findings generated using the above protocol.

RELATED PRODUCTS
1. Protease Arrest™ (Cat. # 786-108): *A cocktail of protease inhibitors for use during protein extraction and purification.*
2. IBS™ (Cat. # 786-183)- *Inclusion bodies solubilization buffer. For solubilization of isolated inclusion bodies prior to re-folding protein procedures.*
3. NI-Protein Assay (Cat. # 786-005): *A protein assay that is free from interference of common laboratory agents, including reducing agents, detergents, dyes, EDTA, etc.*

NOTE: *For other related products, visit our web site at www.GBiosciences.com or contact us.*