PRODUCT INFORMATION

Protein Right Folding Kit

Product Information for PRF-0080:

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. Reason 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 Right Folding 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. 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

Foldase and the agents included in this kit are compatible with many downstream applications. 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.

Contents Protein Folding

[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

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.

Preparation before Use

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

Inclusion Body Solubilization:

1 Isolate the inclusion body.

2 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.

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Protein Right Folding Kit

3 Incubate the inclusion bodies suspension for 1 hour at 4°C.
4 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.

Inclusion Body Solubilization:

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7 Incubate the inclusion bodies suspension for 1 hour at 4°C.
8 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 4-8oC) unless indicated otherwise.
1 Solubilize the inclusion bodies in IBS-Buffer, as described above.
2 Determine the protein concentration of solubilized inclusion bodies. 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 (25µl/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 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 is to help you to get start. For further optimization, the key parameters that need to be adjusted are protein concentration, the residual IBS-Buffer concentration, the time of incubation, and the temperature.

STORAGE CONDITIONS

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

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