Vectrase™-P

Application Guide

Protein production by recombinant methods in *E. coli* or other bacterial systems often results in unfolded or misfolded proteins forming insoluble aggregates known as inclusion bodies. There is a definite advantage to inclusion bodies since they are easy to isolate from cytoplasmic proteins by centrifugation, thus simplifying the purification procedure. Once the inclusion bodies have been isolated, they must be solubilized (denatured) and refolded into the native, active state of the protein.

Proteins that possess disulfide bonds present additional challenges, since the folding of the protein is dependent upon correct disulfide bond formation. Disulfides formed between the wrong residues lead to aggregated or misfolded proteins which are generally inactive.

Vectrase™-P is a proprietary small molecule that assists in the proper folding of proteins, particularly in promoting the reshuffling of incorrect disulfide bonds *in vitro* to obtain the native protein.

How to Use:

***In Vitro* Refolding**

Vectrase™-P is a useful reagent to promote correct folding of proteins that possess disulfide bonds. Carry out the inclusion body solubilization procedure as usual, using a denaturant and/or reducing agent. The presence of dithiothreitol (DTT) or other reducing agents may interfere with the next step of protein refolding using *Vectrase™-P*. It may be necessary to remove them before proceeding to refolding[1].

Prepare a stock of *Vectrase™-P* as 100 mM (26 mg/mL) in methanol. Apply *Vectrase™-P* to your refolding buffer at a final concentration of 1 mM (0.26 g/L). Perform protein folding according to your preferred refolding protocol. It may be necessary to optimize the concentration of *Vectrase™-P* per protein for maximum refolding yield [2, 3].

Notes:

1. BioVectra also offers Immobilized DTT for reducing disulfide bonds in protein denaturation and easy removal by filtration for subsequent steps. Contact BioVectra for details.

2. Optimal yield of refolded protein will require manipulating different components of the mixture. If you have already determined optimal folding conditions for unassisted folding, these can be a good starting point.

3. See reference: Woycechowsky KJ, Wittrup KD, Raines RT: *A small-molecule catalyst in protein folding in vitro and in vivo*. Chemistry & Biology. December 1999, **6**: 871-879.

Cautions:

The folding procedure outlined above is intended as a guideline only and may require modifications to be effective with some proteins.

The optimal ratio of the redox couple (glutathione:oxidized glutathione) in the refolding buffer is protein-specific. Common ratios used in the folding of proteins are 10:1, 5:1, 2:1, or 1:1, respectively. If known, the optimal ratio for unassisted folding by dilution for a protein is a good starting point. *Vectrase™-P* is a reducing agent, therefore, the optimal ratio of glutathione:oxidized glutathione with *Vectrase™-P* may change compared with the optimal ratio in the absence of *Vectrase™-P*.

Time for optimal folding yields may vary significantly from protein to protein; from a few hours to several days. Be sure to allow time for maximum folding activity.

***In Vivo* Folding**

Vectrase™-P has been successfully used as an additive in yeast medium to increase the protein secretion of the heterologous gene of *Schizosaccharomyces pombe* acid phosphatase in *Saccharomyces cerevisiae*. Depending on the cell type and target gene, the concentration and incubation time of *Vectrase™-P* used in the growth medium vary and may require optimization in a case-by-case basis[1].

Vectrase™-P may be added as a supplement from a 100 mM stock (26 mg/mL in dimethyl sulfoxide) to the cell growth medium to a final concentration of 0.1, 0.2 or 0.4 mg/mL. Cells are grown, harvested and treated as usual according to the preferred procedure of the user. Compare the expression yields of the target protein from the test set to determine the optimal concentration of *Vectrase™-P* for *in vivo* use.

Notes:

1. See reference: Woycechowsky KJ, Wittrup KD, Raines RT: *A small-molecule catalyst in protein folding in vitro and in vivo*. Chemistry & Biology. December 1999, **6**: 871-879.

Handling and Storage:

Store *Vectrase™-P* at 2-8°C under argon or nitrogen.

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Targeted Biopharmaceutical Solutions

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