QuickFold™

Protein Refolding Kit

The QuickFold™ Protein Refolding Kit employs a fractional factorial matrix design that allows the researcher to screen for protein refolding in 15 different buffers. The wider range of conditions tested using a stastical design pinpoints the critical factors for refolding in a single experiment. Identification of optimum refolding conditions is then simplified by reducing the number of factors that need to be evaluated. Refolding conditions can be determine in 1 hour.

Kit Components:

- 15 Pre-made Refolding Buffers
- DTT
- Reduced Glutathione
- Oxidized Glutathione
- Sufficient for 11 reactions

AthenaES®

Athena Enzyme Systems™
1-888-892-8408
1450 S. Rolling Rd. Baltimore, MD 21227
www.athenaes.com

Technical Brief:

An Algorithm for Protein Refolding

Sheldon E. Broedel, Jr., Ph. D.
Athena Environmental Sciences, Inc., Baltimore , MD

Athena Enzyme Systems™
1450 South Rolling Road
Baltimore, MD 21227
www.athenaes.com

An Algorithm for Protein Refolding

March 2005

With the extensive array of different approaches which have been reported for the refolding of proteins, it can be a challenge to decide which approach to pursue for any given protein. While in silico models may be helpful in identifying possible solutions, they are not yet sufficiently refined to predict the best conditions for refolding a protein. Therefore, an empirical approach remains the most reliable path to defining the refolding conditions.

There are several parameters which affect the folding of proteins. Most notable are the buffer composition, which includes: (1) Protein concentration, type of buffer, pH, ionic strength, presence of excipitents, and the redox potential, (2) physical parameters such as temperature and time, and (3) the method for refolding, for example using dilution, dialysis, chromatographic, and immobilization techniques either with a single- or double-step buffer exchange. The number of possible combinations is consequently quite high making it impossible to test all of the permutations. To assist in defining the conditions for the refolding of a protein, we have devised an algorithm for screening and then optimizing the method. The strategy is to quickly identify the most likely means for refolding the target protein by first identifying a working buffer system and then eliminating those approaches which are not yielding refolded protein.

The basic scheme for the refolding algorithm is shown on the following page. The scheme relies on a buffer screen which is designed to identify the critical components for proper refolding. A graded set of tests are performed beginning with a simple refolding-by-dilution method and progresses to a more technically difficult refolding-by-chromatography method. In the first round of testing, a set of 15 buffers is used in a single-step refolding by dilution experiment. This is the quickest and simplest method for identifying a suitable buffer and the critical components. Since it is more amenable to a high throughput format, those buffers which do not work can be ruled out early in the process. This makes subsequent experiments with alternative refolding techniques more manageable. Using a subset of buffers, the refolding is repeated by modulating the physical parameters and then testing the potential utility of the different refolding techniques, i.e., standard dialysis, slow dialysis (buffer exchange at 1 ml/min over 48 hours), and chromatographic (affinity, ion exchange or size exclusion). Once the critical parameters for refolding have been identified, the buffer and physical parameters are optimized using a range-finding experimental design followed by a more narrow titration of the specific reagents/conditions to determine the optimum refolding.

There are cases were a single-step refolding technique does not work. In such situations, it is advised that a two-step refolding method be tested. The two-step refolding approach typically uses a dilution technique. In the first step the denatured protein is diluted into a buffer containing a detergent. The choice of detergent is protein-specific and must be determined empirically. After a period of time, one of several different types of cyclodextrins is added to sequester the detergent. Again, the choice of cyclodextrin is protein-specific. The critical buffer composition and physical parameters can be identified using a high throughput screen format with a dilution method. The process is then optimized, as described above, for refolding by dilution, dialysis or chromatography depending on the needs of the protein.

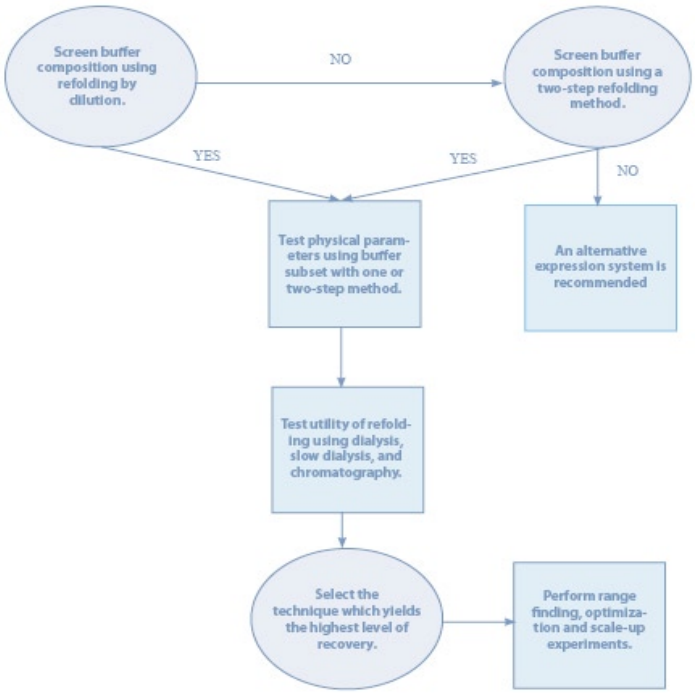

Figure 1. Flow chart of an algorithm to optimize protein refolding.

The initial step is to screen several buffer compositions to identify the critical factors affecting the refolding of a target protein. A one or two-step procedure may be needed depending on the protein. Once the basic buffer composition is defined the physical parameters affecting refolding are determined. To permit scaling of the refolding alternative techniques such as slow dialysis, diafiltration, and immobilization are tested and the most suitable selected. Finally, the system is optimized and scaled to the level needed.