

Pro-Matrix™ Protein Refolding Kit

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89867

1453.0

Number	Description
89867	<p>Pro-Matrix™ Protein Refolding Kit, contains sufficient components to conduct 100 refolding experiments (1 ml each)</p> <p>Kit Contents:</p> <p>Base Refolding Buffer 1, 10 ml; 55 mM Tris, 21 mM NaCl, 0.88 mM KCl; pH 8.2</p> <p>Base Refolding Buffer 2, 10 ml; 440 mM L-arginine, 55 mM Tris, 21 mM NaCl, 0.88 mM KCl; pH 8.2</p> <p>Base Refolding Buffer 3, 10 ml; 880 mM L-arginine, 55 mM Tris, 21 mM NaCl, 0.88 mM KCl; pH 8.2</p> <p>Base Refolding Buffer 4, 10 ml; 550 mM guanidine, 55 mM Tris, 21 mM NaCl, 0.88 mM KCl; pH 8.2</p> <p>Base Refolding Buffer 5, 10 ml; 550 mM guanidine, 440 mM L-arginine, 55 mM Tris, 21 mM NaCl, 0.88 mM KCl; pH 8.2</p> <p>Base Refolding Buffer 6, 10 ml; 550 mM guanidine, 880 mM L-arginine, 55 mM Tris, 21 mM NaCl, 0.88 mM KCl; pH 8.2</p> <p>Base Refolding Buffer 7, 10 ml; 1.1 M guanidine, 55 mM Tris, 21 mM NaCl, 0.88 mM KCl; pH 8.2</p> <p>Base Refolding Buffer 8, 10 ml; 1.1 M guanidine, 440 mM L-arginine, 55 mM Tris, 21 mM NaCl, 0.88 mM KCl; pH 8.2</p> <p>Base Refolding Buffer 9, 10 ml; 1.1 M guanidine, 880 mM L-arginine, 55 mM Tris, 21 mM NaCl, 0.88 mM KCl; pH 8.2</p> <p>Dithiothreitol (DTT), approximately 100 mg*</p> <p>Reduced Glutathione (GSH), approximately 120 mg*</p> <p>Oxidized Glutathione (GSSG), approximately 100 mg*</p> <p>10 mM Polyethylene Glycol (PEG), 1 ml</p> <p>Divalent Cation Stock, 0.5 ml; 400 mM MgCl₂, 400 mM CaCl₂</p> <p>5 M NaCl, 1 ml</p> <p>100 mM EDTA, 1 ml</p> <p>Refolding Guide, 1 instruction manual</p>

*Note: Dry components are dispensed by volume, and not by weight.

Storage: Upon receipt store at -20°C. Product may be stored at 4°C for up to two months. Product is shipped on dry ice.

This product is guaranteed for one year from the date of purchase when handled and stored properly.

Refolding Guide Contents

- Section 1. Matrix Design
- Section 2. Matrix Conditions and Additives
- Section 3. Refolding
- Section 4. Analysis of Folding Reactions
- Appendix A. Inclusion Body Isolation
- Appendix B. Inclusion Body Solubilization
- Appendix C. Analysis of Solubilized Inclusion Bodies
- Appendix D. Purification of Solubilized Inclusion Bodies

Introduction

The Pro-Matrix™ Protein Refolding Kit simplifies and improves the initial stages of developing a refolding protocol without unnecessary trials and data analysis. This kit uses select reagents and conditions proven effective for the high-yield refolding of many proteins in a three-level adjustable matrix format that allows both screening and optimization of folding conditions.

The overexpression of eukaryotic proteins in transformed microorganisms often results in the accumulation of inactive and improperly folded recombinant proteins in insoluble aggregates called inclusion bodies. In many cases it is possible to solubilize and refold an aggregated protein to its native state. While several approaches to protein refolding have been developed, the use of small molecule buffer additives is the most common method for initial refolding experiments because of historical success with a variety of proteins and the relatively simple and inexpensive protocol.¹⁻⁴ For buffer-based refolding methods, inclusion bodies are first isolated, purified, and then solubilized with a strong denaturant, such as guanidine hydrochloride (GdnHCl). The solubilized protein is then diluted or dialyzed into a refolding buffer to reduce the denaturant concentration and the protein refolds based on the information contained in its primary sequence. If the denaturant is removed and replaced with a non-optimized refolding buffer, protein aggregation strongly competes with renaturation and minimal amounts of native protein are recovered.

The degree of aggregation that occurs during refolding is largely dependent on protein concentration, concentration of strong and weak denaturants, pH, temperature and redox environment. Ionic strength, divalent cations, polymers and cofactors can also promote refolding of some proteins. Each of these conditions will interact in positive or negative ways that are unique to each target protein. Therefore, selection and optimization of buffer conditions must be empirically determined, which can be difficult and time-consuming, requiring extensive trial and error and literature research.

Fractional matrices provide the most efficient means for screening refolding conditions with a limited number of samples and materials;^{5,6} however, experiments using fractional matrices can have significant limitations based on their design. Typically, fractional matrices are designed to examine many factors simultaneously using two test levels for each factor. This type of design often results in folding conditions that are far from optimal, producing low refolding yields and generating data that provides only minimal information for optimization. Furthermore, matrix-based experimental designs can often force chemical combinations that are not useful as refolding solutions. The Pro-Matrix™ Protein Refolding Kit is based on a fractional factorial matrix, but it has been designed to minimize limitations and provide the best chance of successfully developing a refolding protocol. The key advantages of this method are as follows:

- The conditions and components examined are limited to those having the most significant and general utility in refolding buffers.
- The matrix design is open and adjustable, allowing for both screening and optimization of refolding conditions.
- Buffer components are screened at three concentration levels to accommodate for wide differences in folding conditions required for different proteins.
- A three-level screen significantly reduces the amount of secondary optimization required and increases the ease of data interpretation.
- Known positive and negative interactions between buffer components are addressed, minimizing unnecessary analyses.

Note:

Thank you for your interest in the Pierce Pro-Matrix™ Protein Refolding Kit. Because of this kit's specific protocol design, an extensive refolding guide is provided with the Pro-Matrix™ Protein Refolding Kit. Instructions may be obtained through the purchase of the Pro-Matrix™ Protein Refolding Kit. Please contact Pierce Biotechnology Customer Service at 1-800-8-PIERCE (1-800-874-3723) or 1-815-968-0747 for further information or to place an order.

Related Pierce Products

22582	Ellman's Reagent, 5 g
23215	Compat-Able™ Protein Assay Preparation Reagent Set, two reagent set with sufficient material to pre-treat up to 500 samples prior to total protein assay.
23236	Coomassie Plus Protein Assay Reagent, 950 ml
24590	GelCode® Blue Stain Reagent, 500 ml
26800	PAGEprep® Protein Enrichment and Clean-up Kit, sufficient reagents for 50 samples
66373	Slide-A-Lyzer® Dialysis Cassette*, 7,000 MWCO 0.1-0.5 ml capacity, 10/pkg
78415	Halt™ Protease Inhibitor, sufficient reagents for 100 ml of extract
77712	Immobilized TCEP Disulfide Reducing Gel, 5 ml
78260	B-PER® II Bacterial Protein Extraction Reagent**, 250 ml
89833	Lysozyme, 5 g
89835	DNase I, 5,000 units
89849	Protein Desalting Spin Columns, 25/pkg

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*Slide-A-Lyzer® Dialysis Cassette Technology is protected by U.S. Patent # 5,503,741.

**B-PER® Technology is protected by U.S. Patent # 6,174,704.

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