INSTRUCTIONS

Pierce® Protein Refolding Kit

89867

1453.2

Number	Description
89867	**Pierce Protein Refolding Kit,** contains sufficient components to conduct 100 refolding reactions (1mL each)

Kit Contents:

Base Refolding Buffer 1, 10mL; 55mM Tris, 21mM NaCl, 0.88mM KCl; pH 8.2

Base Refolding Buffer 2, 10mL; 440mM L-arginine, 55mM Tris, 21mM NaCl, 0.88mM KCl; pH 8.2

Base Refolding Buffer 3, 10mL; 880mM L-arginine, 55mM Tris, 21mM NaCl, 0.88mM KCl; pH 8.2

Base Refolding Buffer 4, 10mL; 550mM guanidine, 55mM Tris, 21mM NaCl, 0.88mM KCl; pH 8.2

Base Refolding Buffer 5, 10mL; 550mM guanidine, 440mM L-arginine, 55mM Tris, 21mM NaCl, 0.88mM KCl; pH 8.2

Base Refolding Buffer 6, 10mL; 550mM guanidine, 880mM L-arginine, 55mM Tris, 21mM NaCl, 0.88mM KCl; pH 8.2

Base Refolding Buffer 7, 10mL; 1.1 M guanidine, 55mM Tris, 21mM NaCl, 0.88mM KCl; pH 8.2

Base Refolding Buffer 8, 10mL; 1.1 M guanidine, 440mM L-arginine, 55mM Tris, 21mM NaCl, 0.88mM KCl; pH 8.2

Base Refolding Buffer 9, 10mL; 1.1 M guanidine, 880mM L-arginine, 55mM Tris, 21mM NaCl, 0.88mM KCl; pH 8.2

Dithiothreitol (DTT), approximately 100mg*

Reduced Glutathione (GSH), approximately 120mg*

Oxidized Glutathione (GSSG), approximately 100mg*

10mM Polyethylene Glycol (PEG), 1mL

Divalent Cation Stock, 0.5mL; 400mM $MgCl_2$, 400mM $CaCl_2$

5 M NaCl, 1mL

100mM EDTA, 1mL

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*__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.

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Refolding Guide Contents

Introduction

The Pierce 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 over-expression 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. Although 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 Pierce 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 Protein Refolding Kit. Because of this kit's specific protocol design, an extensive refolding guide is provided with the kit. Instructions may be obtained through kit purchase. Please contact Customer Service at 1-800-874-3723 or 1-815-968-0747 for further information or to place an order.

Related Thermo Scientific Products

22582	**Ellman's Reagent,** 5g
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 (Bradford) Protein Assay Reagent,** 950mL
24590	**GelCode™ Blue Stain Reagent,** 500mL
89888	**Pierce SDS-PAGE Sample Prep Kit**
66373	**Slide-A-Lyzer® Dialysis Cassette,** 7,000 MWCO 0.1-0.5mL capacity, 10/pkg
87785	**Halt Protease Inhibitor Cocktail, EDTA-Free** (100X), 1 ml
87786	**Halt Protease Inhibitor Cocktail,** contains sufficient reagents to treat 100 ml of sample
77712	**Immobilized TCEP Disulfide Reducing Gel,** 5mL
78260	**B-PER® II Bacterial Protein Extraction Reagent,** 250mL
89833	**Lysozyme,** 5g
89835	**DNase I,** 5,000 units
89849	**Protein Desalting Spin Columns,** 25/pkg

Cited References

1. Kelly, R.F. and Winkler, M.E. (1990). Folding of eukaryotic proteins produced in *Escherichia coli*. *Genetic Engineering.* **12:**1-19.
2. Rudolph, R. and Lilie, H. (1996). In vitro folding of inclusion body proteins. *FASE.* **10:**49-56.
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4. Middelberg, A.P.J. (2002). Preparative protein refolding. *Trends Biotechnol.* **20(10):**437-43.
5. Ahn, J.H., *et al.* (1997). Investigation of refolding condition for *Pseudomonas fluorescens* lipase by response surface methodology. *J. Biotechnol.* **54:**151-60.
6. Armstrong, N., *et al.* (1999). A new protein folding screen: Application to the ligand binding domains of glutamate and kainate receptor and to lysozyme and carbonic anhydrase. *Protein Science* **8(7):**1475-83.

General References

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Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

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