Novagen®

iFOLD™ Protein Refolding System 1

Inclusion body preparation and a 96-well matrix of buffers and additives for convenient optimization of refolding conditions

Overexpression of recombinant proteins in *E.coli* is an efficient method for producing large quantities of target proteins for many biological and new drug development applications. However, proteins often accumulate in the cells as inactive, misfolded, and insoluble aggregates (inclusion bodies). Consequently, the target protein must be properly refolded to regain activity.

The iFOLD™ Protein Refolding System1 is a simple, reliable, and comprehensive method to identify optimal protein refolding conditions. The system includes inclusion body purification reagents and a 96-well plate-based protein refolding buffer matrix (92 experimental and 4 control wells).

Features:

- Includes reagents for inclusion body purification
- Comprehensive 96-well protein refolding buffer matrix
- pH range (7.0 - 8.5)
- Refolding additives include salts, cyclodextrin, redox agents chaotropes and glycols
- Refolding conditions based on extensive literature review and REFOLD database (http://refold.med.monash.edu.au)
- High-throughput compatible

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iFOLD™ Protein Refolding System 1

Structural and functional proteomics require large amounts of very pure and correctly folded protein. Due to simplicity, low cost, and potential to express large quantities of target protein, *E. coli* expression systems are frequently used. However, production of foreign proteins in *E. coli* may result in the formation of inclusion bodies. Although inclusion body formation is typically not desirable, the aggregates are resistant to proteolysis, easily purified, and can be denatured with chaotropes. Unfortunately, defining conditions that promote refolding of the target protein into its native conformation is empirical and difficult. The iFOLD™ Protein Refolding System 1 is designed to determine optimal protein refolding conditions by a systematic evaluation of 92 different buffers, containing unique combinations of pH, salt, cyclodextrin, redox agent, and refolding additives. The iFOLD system buffer matrix is based on an extensive literature review of successful refolding experiments and information contained in the REFOLD database (http://refold.med.monash.edu.au). The system includes inclusion body purification reagents combined with a dispensed 96-well plate-based protein refolding buffer matrix. Following cell lysis, membrane components and contaminating proteins trapped within inclusion body pellets are removed by a series of detergent and buffer washes. The purified inclusion bodies are denatured by addition of TCEP [Tris(2-carboxyethyl)phosphine] and N-Lauroylsarcosine and refolded by rapid dilution into the iFOLD 96-well buffer matrix. Each kit is sufficient for screening up to 96 refolding conditions (92 experimental and 4 control wells) for a single protein.

Features:

- All reagents for inclusion body purification and pre-dispensed iFOLD 96-well protein refolding buffer matrix
- Comprehensive set of optimized screening conditions for protein refolding
- Refolding conditions based on extensive literature review
- 96-well refolding plate compatible with HT methodology

iFOLD™ Protein Refolding System 1 plate layout

Brief Protocol

Express target protein

Harvest cells

Lyse cells by sonication

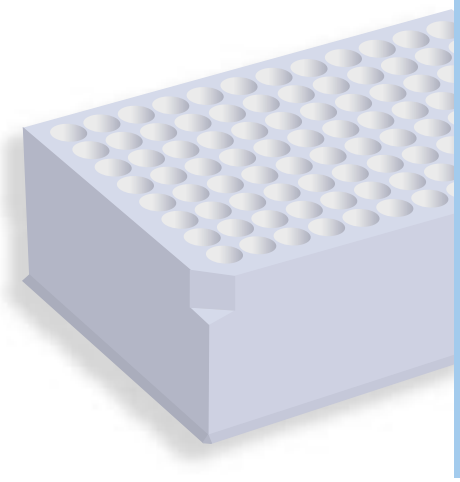
Centrifuge to pellet inclusion bodies

Wash inclusion bodies

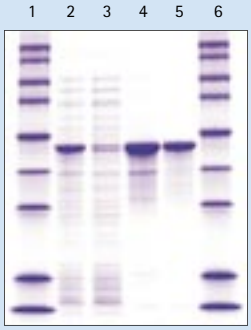
Denature inclusion bodies with N-Lauroylsarcosine

Refold target protein by rapid dilution into iFOLD™ Protein Refolding System 1 buffer matrix

Assay for correctly folded and active target protein



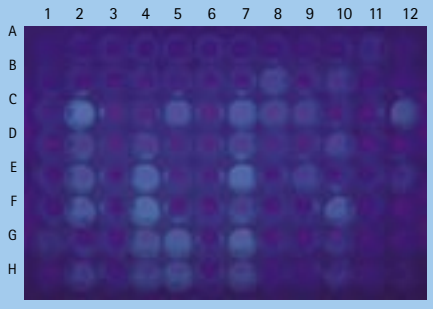
Purification of thioredoxin-GFP fusion protein from inclusion bodies



Lane	Sample
1	Perfect Protein™ Markers 10-225 kDa
2	Total cellular protein
3	Soluble fraction
4	Insoluble fraction
5	Purified inclusion body fraction
6	Perfect Protein Markers 10-225 kDa

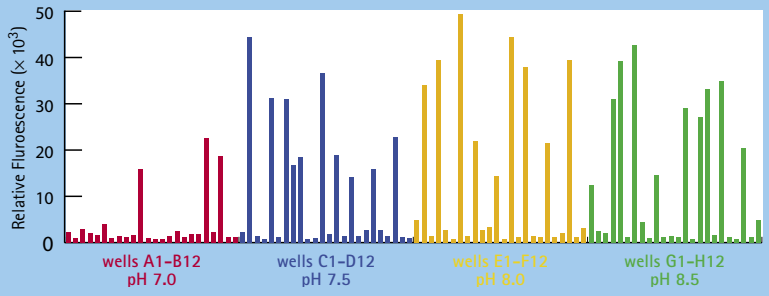
A pET vector carrying a thioredoxin-GFP cDNA was transformed and expressed in *E. coli*. Thioredoxin-GFP inclusion bodies were purified following the iFOLD™ Protein Refolding System 1 protocol. Samples were analyzed by SDS PAGE (4-20% gradient gel, Coomassie blue staining)

Refolded thioredoxin-GFP fusion protein



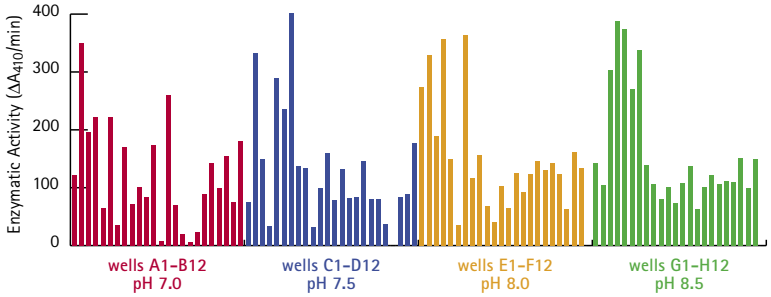
Thioredoxin-GFP fusion protein refolded with the iFOLD™ Protein Refolding System 1. The iFOLD plate was illuminated with a standard UV lightbox and photographed with a digital camera.

Relative fluorescence of a thioredoxin-GFP fusion after refolding



Thioredoxin-GFP Inclusion bodies were isolated and purified according to the iFOLD Protein Refolding System 1 protocol. Thioredoxin-GFP was refolded by rapid dilution of the N-Lauroylsarcosine-denatured protein into the iFOLD plate. Relative fluorescence of the samples was recorded approximately 20 h after addition of the sample to the iFOLD plate.

Catalytic activity of λ phosphatase after refolding



λ phosphatase Inclusion bodies were isolated and purified according to the iFOLD Protein Refolding System1 protocol. λ phosphatase was refolded by rapid dilution of the N-Lauroylsarcosine-denatured protein into the iFOLD plate, followed by a 20 h incubation at room temperature. Catalytic activity of λ phosphatase was measured by recording cleavage of 4-nitrophenyl phosphate at 30°C.

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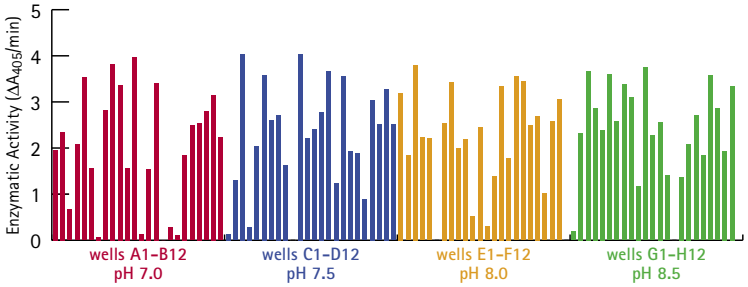
Catalytic activity of HRV 3C protease after refolding



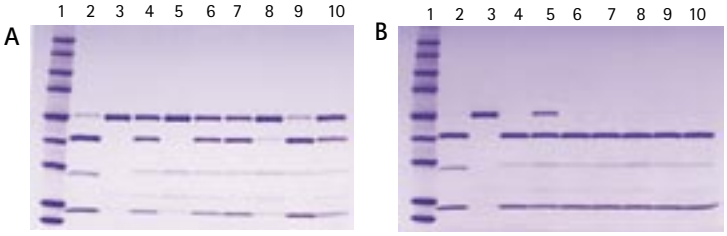
HRV 3C Inclusion bodies were isolated and purified according to the iFOLD™ Protein Refolding System 1 protocol. HRV 3C was refolded by rapid dilution of the denatured protein into the iFOLD plate, followed by a 20 h incubation at room temperature. Catalytic activity of HRV 3C was measured by recording cleavage of a peptide substrate (H-Glu-Ala-Leu-Phe-Gln-*p*NA; Bachem) at 30°C.

Product	Cat. No.
iFOLD™ Protein Refolding System 1	71552-3

Components:

- 30 ml 10X IB-Prep™ Buffer
- 0.5 ml 1M TCEP
- 1.5 ml Triton® X-100
- 0.1 ml Lysonase™ Bioprocessing Reagent
- 10 ml 30% N-Lauroylsarcosine
- 50 ml 50X iFOLD Dialysis Buffer
- 1 iFOLD Protein Refolding Plate 1
- 2 Aluminum Plate Sealers

Cleavage of the control protein by HRV 3C protease after refolding



Samples with low, moderate, or high catalytic activity against the H-Glu-Ala-Leu-Phe-Gln-pNA peptide were tested for cleavage of an HRV 3C cleavage control protein. Reactions were performed at 4°C for 30 min (panel A) or 17 h (panel B) and then analyzed by SDS PAGE (4–20% gradient gel, Coomassie blue staining).

Lane	Sample
1	Perfect Protein™ Markers, 10–225 kDa
2	Native HRV 3C protease (positive control)
3	No protease (negative control)
4	iFOLD well A9
5	iFOLD well B5
6	iFOLD well C6
7	iFOLD well D4
8	iFOLD well E6
9	iFOLD well F10
10	iFOLD well G10

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