

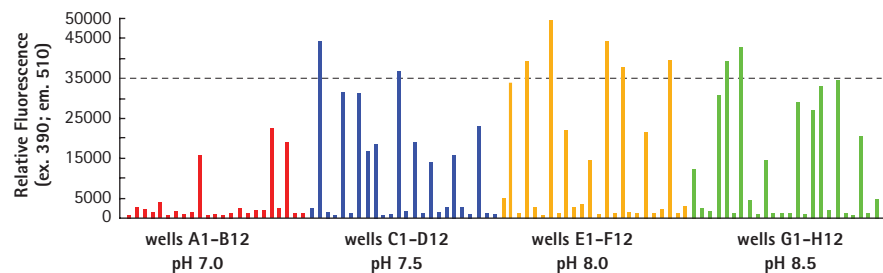
# Large-scale refolding of trx-GFP following an iFOLD™ Protein Refolding System 1 screen

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Overexpressing recombinant proteins in *E. coli* is an efficient method to produce large quantities of target proteins. However, foreign proteins expressed in *E. coli* may accumulate as inclusion bodies (IB), which are dense, insoluble aggregates of misfolded protein. To gain native conformation, the target protein must be denatured and correctly folded. The iFOLD™ Protein Refolding System 1 includes all reagents needed to prepare and denature IB, and a 96-well plate containing 92 different refolding buffers, plus 4 control wells, to efficiently determine optimal refolding conditions (Leland 2006). After an optimal refolding buffer has been identified, the protein refolding reaction can be easily scaled up

## iFOLD Protein Refolding System 1

In part, the iFOLD System 1 is based on an artificial chaperone system (Rozema 1995) using *N*-lauroylsarcosine (LS), which is a chaotropic anionic detergent, and methyl- $\beta$ -D-cyclodextrin, which acts as a detergent sponge. LS coats hydrophobic segments of the target protein, solubilizing inclusion bodies (Frankel 1991). The cyclodextrin, present in some of the refolding buffers, strips the LS from the target protein, allowing the protein to refold. For some proteins, low concentrations of LS promote, rather than inhibit, successful refolding, so not all wells contain cyclodextrin. The remaining components and pH range used for the iFOLD Protein Refolding Plate 1 were determined by fractional



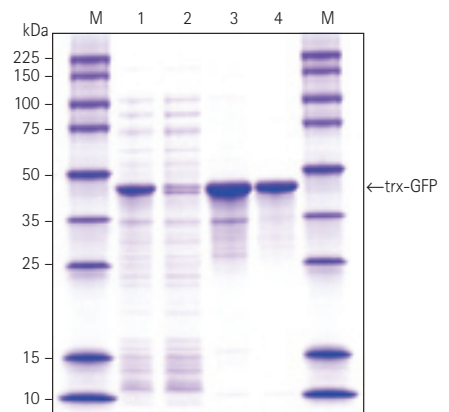
**Figure 1. Refolding screen for trx-GFP**

Samples from an iFOLD trx-GFP refolding screen were diluted 1:4 with 50 mM Tris-HCl, pH 8.0, and the relative fluorescence intensity was measured (excitation 390 nm; emission 510 nm).

factorial and rational design after an extensive review of successful refolding experiments reported in literature and in the REFOLD database (<http://refold.med.monash.edu.au>) (Vincentelli 2004, Willis 2005, Chow 2005). The 96-well iFOLD Protein Refolding Plate is convenient for bench-top use and is compatible with high-throughput robotic systems.

## Scale-up

Here, we report the large-scale refolding of a thioredoxin-green fluorescent protein (trx-GFP) fusion based on conditions identified from an iFOLD System 1 refolding screen. The iFOLD System 1 screen identified several buffer conditions that supported successful refolding of trx-GFP. Every protein refolding solution resulting in relative fluorescence intensity >35,000 (Figure 1) contained cyclodextrin, which sequesters LS, indicating that residual LS is detrimental to refolding trx-GFP. The buffer composition of well E7 (50 mM Tris-HCl,



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

**Figure 2. SDS-PAGE of trx-GFP inclusion body isolation using the iFOLD Protein Refolding System 1**

4–20% gradient gel, Coomassie blue staining.

pH 8.0, 250 mM NaCl, 12.5 mM cyclodextrin, 1.0 mM Tris[2-carboxyethyl] phosphine [TCEP]) was selected for use in the large-scale refolding experiment.

## Method

### IB preparation

A cDNA coding for GFP was cloned into pET-32 Ek/LIC and was expressed as trx-GFP in BL21(DE3). Cells were grown and autoinduced in Overnight Express™ Instant TB Medium overnight at 37°C. The culture was centrifuged and the resulting cell pellet was processed using iFOLD™ System 1 reagents and protocol. Reagent volumes for cell lysis and IB preparation were scaled appropriately from the initial screen (Figure 2) and yielded 1.72 g trx-GFP IB. IB were denatured and reduced (Table 1, Figure 3) using the detergent, LS (Lauroylsarcosine, Sodium Salt, Cat. No. 428010) combined with the reducing agent TCEP (TCEP, Hydrochloride, Cat. No. 580560).

### Refolding

Using 504 mg of the denatured trx-GFP, the protein was refolded in 5.0 L of well E7 refolding buffer. The refolded sample was concentrated to approximately 500 ml using an ultrafiltration membrane with a 10-kDa molecular weight limit (Table 1, Figure 3). After ultrafiltration some precipitate formed, possibly misfolded protein, accounting for a portion of the sample loss at that step (Table 1). The refolding buffer was exchanged to a storage buffer containing 10% (v/v) glycerol and 0.03% (w/v) BRIJ® 35 detergent to stabilize the protein.

### Conclusion

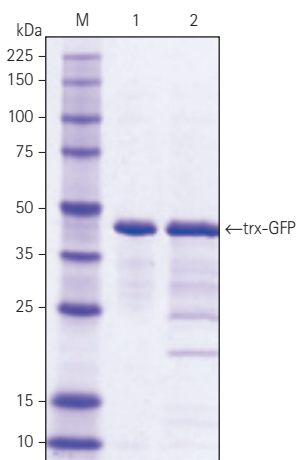
A single screen with the iFOLD Protein Refolding System 1 identifies the distinct factors important for refolding a specific protein, so that an optimal buffer can be prepared for the large scale refolding reaction. The iFOLD procedure is easily scaled up to purify and denature inclusion bodies and provide high yields of correctly folded proteins for downstream applications. ■

**Table 1. Trx-GFP refolding and purification yields**

Stage	Sample mass*	RFU/mg protein
IB pellet	1.72 g	n/a
Denatured and dialyzed IB pellet	504 mg	25
After refolding	500 mg	250,000
After concentration and buffer exchange	269 mg	5,500,000

\* Protein concentrations were determined using the BCA Protein Assay Kit (Cat. No. 71285) with BSA as a standard. Fluorescence intensity data were collected using a Tecan® Safire™ fluorescence plate reader

n/a: not available; RFU: relative fluorescence units



Lane	Sample
M	Perfect Protein™ Markers 10-225 kDa
1	Denatured trx-GFP inclusion bodies, 3 µg total protein
2	Refolded trx-GFP after ultrafiltration, 3 µg total protein

**Figure 3. Scale-up of trx-GFP refolding following an iFOLD Protein Refolding System 1 screen**

Product	Size	Cat. No.	Price
iFOLD™ Protein Refolding System 1	1 system	71552-3	\$350
pET-32 Ek/LIC Vector Kit	20 rxn	69076-3	\$379
BL21(DE3) Competent Cells	0.4 ml 1 ml	69450-3 69450-4	\$73 \$134
Overnight Express™ Instant TB Medium	1 EasyPak (1 x 60 g) 5 EasyPaks (5 x 60 g)	71491-3 71491-4	\$36 \$144
	1 kg	71491-5	\$355
Lauroylsarcosine, Sodium Salt	5 g	428010	\$139
TCEP, Hydrochloride	1 g	580560	\$48
BCA Protein Assay Kit	500 assays	71285-3	\$95

### Components

#### Cat. No. 71552

- 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

### REFERENCES

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