**BENCHMARKS**

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Expression of foreign proteins in *E. coli* is frequently stymied by the formation of insoluble inclusion bodies. To recover the insoluble protein, the inclusion body is usually solubilized with a denaturing reagent such as urea and guanidine HCl, followed by dialysis to renature the target protein. Nondetergent sulfobetaines (NDSB) are a family of zwitterionic compounds carrying a hydrophobic group that is not long enough for micelle formation. Recent modifications of existing methods.

**Table 1. Representative Yield of Functional GST-C/EBP**

<table>
<thead>
<tr>
<th></th>
<th>GST-C/EBP</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total expressed protein</td>
<td>2200 µg</td>
<td>100%</td>
</tr>
<tr>
<td>Renatured protein</td>
<td>1125 µg</td>
<td>51.1%</td>
</tr>
<tr>
<td>Functional protein</td>
<td>1047.3 µg</td>
<td>47.6%</td>
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aExpression is performed in a 500-mL culture.

bProtein yield relative to the total expressed protein.

GST-C/EBP was placed downstream of the GST fragment in pGEX5T-1. The resultant construct was transformed into BL21(DE3)pLysS for protein expression. A positive colony was inoculated into 25 mL LB medium containing 100 µg/mL ampicillin and incubated at 37°C overnight. Next day, the saturated culture was placed into 500 mL culture medium and incubated at 37°C until the cell density reached A600 = 0.8. Protein expression was induced with 0.5 mM IPTG at 37°C for 2 h. The bacteria were spun down and resuspended in the lysis buffer (50 mM Tris, pH 7.5, 250 mM NaCl, 1% Triton X-100, and 5 mM DTT). Cells were lysed by one cycle of freezing and thawing plus a brief sonication in the presence of the complete, EDTA-free protease inhibitor cocktail (Roche Molecular Biochemicals, Mannheim, Germany). The lysate was then treated with 20 µg/mL DNase I at 37°C for 1 h. The insoluble inclusion body was spun down and washed twice with 1% Triton X-100. The inclusion body was dissolved in the denaturation solution (6 M guanidine HCl, 25 mM DTT, and 50 mM Tris, pH 7.5) at 4°C for 1 h. The protein concentration of solubilized sample was then adjusted to 1 µg/µL with the denaturation solution. For renaturation, the solubilized sample was quickly diluted with a tenfold volume of renaturation solution (1 M NDSB, 0.2 M NaCl, 1 mM DTT, and 50 mM Tris, pH 7.5) at 4°C for 1 h. The renatured sample was dialyzed against double-distilled water at 4°C overnight. The dialyzed sample was concentrated with PEG 20,000 and purified with glutathione agarose. The purified GST-C/EBP fusion protein was eluted with the elution buffer (20 mM reduced glutathione, 120 mM NaCl, and 100 mM Tris, pH 8.0), dialyzed against 20 mM Tris (pH 7.5), and stored at -20°C.

We tested the efficiency of NDSB 201 in the renaturation of GST-C/EBP. We reasoned that correctly folded GST-C/EBP protein will bind to the glutathione agarose beads and be eluted with the reduced glutathione. As shown in Figure 1A, the eluted GST-C/EBP analyzed by SDS-PAGE appeared to match the predicted size. To further test the bioactivity of the renatured GST-C/EBP, we performed the electrophoretic mobility shift assay (EMSA) and the GST pull-down assay. For EMSA, a C/EBP responder element (FP1, 5'-TTGTGTC-TCAACATGTGTA-3') derived from the rat pregnancy-specific glycoprotein gene, *rnCGM3*, was used as the probe (3). The radiolabeled FP1 fragment was incubated with C/EBP translated in vitro and the renatured GST-C/EBP, respectively. As shown in Figure 1B, lanes 3 and 7, a specific DNA-protein complex was formed with both protein samples because unlabeled FP1 could compete with complex formation (lanes 2 and 6). Moreover, antibody specific to C/EBP was able to abolish the formation of the complex between FP1 and the C/EBP translated in vitro or the renatured GST-C/EBP (Figure 1B, lanes 4 and 8). Antibody specific to GST was also able to prevent the formation of the FP1-GST-C/EBP complex (Figure 1B, lane 5). Because C/EBP interacts with the p50 subunit of NFkB (5), we also performed pull-down assays using the renatured GST-C/EBP and 35S-methionine-incorporated p50. As shown in Figure 1C, p50 specifically interacted with GST-C/EBP (lane 3) but not GST (lane 2). These results suggested that preparation of active GST-C/EBP from inclusion body has been improved.

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**Preparation of Functional Recombinant Protein from *E. coli* Using a Nondetergent Sulfobetaine**

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bodies is facilitated with NDSB 201. Table 1 shows the yield from a 500-mL culture. As shown, the yield of the functional GST-C/EBP\textsuperscript{b} is 47.6% of the total expressed proteins. We have also compared the efficiency of NDSB 201 with other NDSBs (NDSB 195 and 256) on the preparation of active GST-C/EBP\textsuperscript{b}. We found that NDSB 195 indeed resulted in a better yield than NDSB 201 or 256 of the correctly folded GST-C/EBP\textsuperscript{b} (data not shown). However, NDSB 201 is sufficient to meet our experimental purposes in terms of cost effectiveness. Therefore, we suggest testing the efficiencies of different NDSBs in the refolding of the protein of interest in a pilot study before a large-scale purification is performed.

REFERENCES


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