Molecular Biology

Lysis of *E.coli* for the Purification of Soluble Recombinant Proteins using CellLytic-B™ and CellLytic-B™ II

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Introduction

The first step in the purification of a cytoplasmic or periplasmic recombinant protein expressed in *E. coli* is the lysis of the cell to release the proteins. Osmotic lysis[1,2] of bacteria can be used for periplasmic expressed proteins but leaves the researcher with a large volume of dilute protein. Sonication, lysozyme or French pressure cell treatments[3] are often used to lyse bacterial cells expressing cytoplasmic proteins. Recovery from these methods is variable and many times the yield is low. Sonication generates heat that may be detrimental to many proteins. Both sonication and French pressure cells require specialized equipment and do not work effectively with large volumes. Lysozyme treatment adds protein to the extract and may introduce unwanted proteolytic enzymes. We have developed a ready-to-use extraction reagent that lyses cells by the use of a gentle detergent. It is formulated in two different strengths to suit the researcher's needs. The standard strength CellLytic-B™ is useful for most applications, while the double strength CellLytic-B™ II produces a more concentrated protein solution.

Materials and Methods

The first experiment was designed to compare different methods of extraction of a recombinant protein. A recombinant clone expressing bacterial alkaline phosphatase (BAP) protein with an N-terminal FLAG tag and a C-terminal poly-histidine (FLAG-BAP-His) was grown in 4 L of EZMix™ Terrific Broth (Product Code: T 9179) for about 7 hours at 37 °C. The cells were then induced with 1 mM isopropyl β-D-thiogalactopyranoside [IPTG] (Product Code: I 6758) and allowed to grow an additional 2 hours. Cells were harvested by centrifugation at 5,000 x g in a J2-21 centrifuge (Beckman Counter, Fullerton, CA). The medium was removed and the cell paste was frozen at –20 °C. One gram of the recombinant *E. coli* cell paste expressing the FLAG-BAP-His was extracted for each of the methods. The CellLytic-B™ (Product Code: B 3553) was used at 10 ml/g of cell paste. The lysozyme treatment was done with 10 mg/ml of lysozyme (Product Code: L 7651) plus 10 mM EDTA (Product Code: E 5134) for 15 minutes on ice. The sonication was done for 2 minutes while incubated on ice. The sonication/lysozyme treatment was performed by first treating the cells with the lysozyme and then sonicating the extract. All of the extracts were clarified by centrifugation for 15 minutes at 20,000 x g at 4 °C. The supernatants were recovered and then analyzed for total protein by the Bradford protein assay (Product Code: B 6916).

In the second experiment, samples from the same cell paste were used with varying amounts of CellLytic-B™ and CellLytic-B™ II (Product Codes: B 3553 and B 3678, respectively). Aliquots of frozen cell paste expressing FLAG-BAP-His protein were extracted using different amounts of CellLytic-B™ per gram of cell paste. One gram of frozen cell paste was extracted using 2.5, 5.0, 10, 20, 30, 40 and 50 ml of CellLytic-B™ per gram of cell paste. The cells were suspended and mixed well for 15 minutes at room temperature. The extracts were centrifuged for 15 minutes at 20,000 x g at 4 °C. The supernatant was removed and placed in a clean tube. Samples were analyzed for total protein by the Bradford protein assay and also assayed for alkaline phosphatase activity using p-nitrophenol phosphate (pNPP).

In the last experiment three different affinity columns were used to purify recombinant proteins. One-ml fractions were collected and run from gravity flow columns. A 10-ml sample of FLAG-BAP-His protein extracted with CellLytic-B™ was applied to a 1-ml column of ANTI-FLAG® M2 resin (Product Code: A 1205). The M2 resin was then washed with 10 ml of Tris buffered saline (TBS; Product Code: T 6664), pH 8.0, to remove all of the unbound protein. The M2 column was eluted with 100 mg/ml FLAG® peptide (Product Code: F 3290) in TBS. Another 10-ml sample of FLAG-BAP-His protein extracted with CellLytic-B™ was applied to a 1-ml column of nickel chelate resin. This column was washed with 15 ml of 50 mM sodium phosphate, pH 8.0, 0.3 M sodium chloride, and 10 mM imidazole. The bound material was eluted with 50 mM sodium phosphate, pH 8.0, 0.3 M sodium chloride, and 250 mM imidazole. The last column was charged with a GST-GRB2 fusion protein grown in a manner identical to that described for the FLAG-BAP-His protein. One gram of the GST-GRB2 cell paste was extracted with 10 ml of CellLytic-B™ as previously described. This extract was loaded onto a 1-ml glutathione column. The column was washed with 10 ml of 50 mM Tris-Cl, pH 8.0. The column was eluted with 5 ml of 50 mM Tris-Cl , pH 8.0, with 10 mM glutathione (Product Code: G 4251). The crude extracts and eluted proteins were analyzed for protein by the Bradford protein assay. The crude extracts and affinity-purified proteins were separated on a 12% SDS-PAGE gel. Twenty-five micrograms of crude extract and 5 µg of purified protein were loaded on the gel. The gel was stained with EZBlue™ Gel Staining Reagent (Product Code: G 1041).

Results and Discussions

The CellLytic-B™ produced the highest amount of total soluble protein per gram of cell paste (Figure 1). The extraction resulted in a 33% increase in the total amount of protein in the same time frame as in the other three methods. The total time is actually less since there was no need for cleaning special equipment and no reagents to make, since CellLytic-B™ is premixed and ready-to-use. The CellLytic-B™ also gives reproducible extracts every time (data not shown). The extracts prepared by sonication may vary from extraction-to-extraction depending upon the extraction volume and sonication time. The minimum volume of CellLytic-B™ necessary for full extraction of protein is about 10 ml/g of cell paste. Higher amounts of reagent do not increase the amount of protein extracted (Figure 2).

CellLytic-B™ II is a more concentrated formula and only required about 5 ml/g of cell paste to produce the same amount of extraction as CellLytic-B™ (10 ml/g of cell paste). A more important measure of the required extraction volume is the amount of recovered target protein. In this case, the target protein was a bacterial alkaline phosphatase. The maximum amount of enzyme extracted followed the same curve as the protein amounts (Figure 3). Using more reagent per gram of cell paste did not increase the amount of extracted protein.

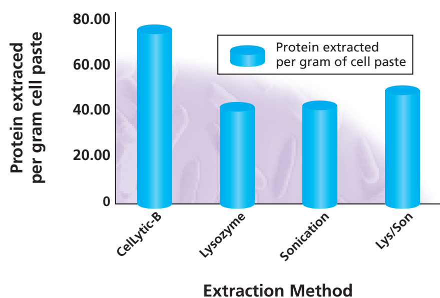

Figure 1. Extraction efficiency. *The bars represent the amount of protein extracted from frozen cells when using CelLytic-B™, lysozyme treatment, sonication or lysozyme and sonication (Lys/Son). The methods are described in the Materials and Methods.*

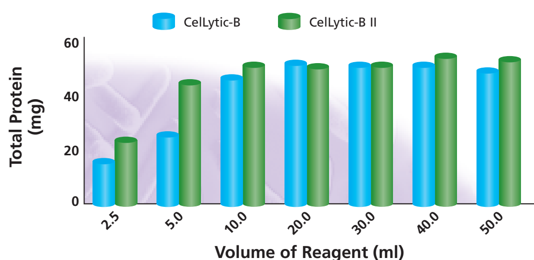

Figure 2. Effect of volume variation on extraction efficiency. *The bars represent the amount of protein extracted from frozen cells when using varying amounts of CelLytic-B™ and CelLytic-B™ II.*

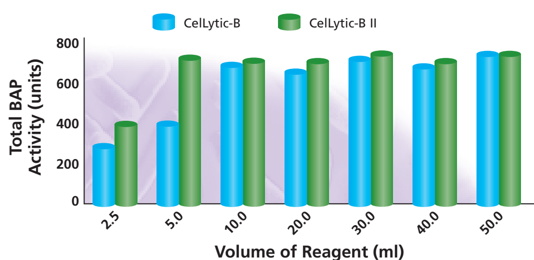

Figure 3. Recovered target protein vs extraction volume. *The bars represent the amount of total enzyme activity (BAP) extracted from frozen cells when using varying amounts of CelLytic-B™ and CelLytic-B™ II.*

The detergent in both reagents did not interfere with the affinity purification tags. The results of GST, metal chelate, and antibody (FLAG® system) chromatography all yield high-purity products in the presence of this reagent (Figure 4). In fact, in many cases the purity will be higher in the presence of the CelLytic-B™ than when using conventional methods. The detergent does need to be removed before any of these affinity chromatography matrices are used. The detergent in CelLytic-B™ and CelLytic-B™-II is known to interfere with the maltose-binding protein system. Using standard dialysis methods to remove the detergent before applying the extraction solution to the column can alleviate this. This CelLytic-B™ extraction method uses a high relative centrifugal force to pellet any released chromosomal DNA. Lower speeds may be used and the viscous DNA may be digested by the addition of nucleases such as DNase I (Product Code: D 4527) or Endonuclease [Benzonase] (Product Codes: E 8263 or E 1014). This will result in a solid pellet and a thin liquid phase.

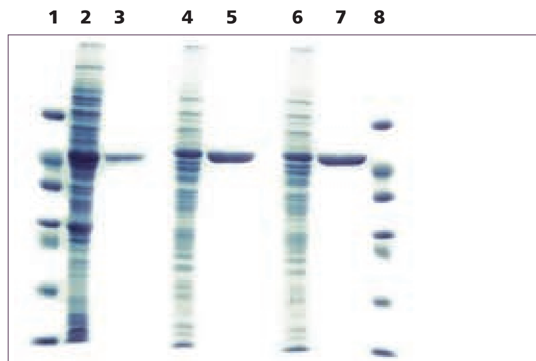

Figure 4. CelLytic-B™ compatibility with affinity chromatography. *SDS-PAGE gel of recombinant cell extracts prepared with CelLytic-B™ and used in affinity purification of GST, FLAG® and Poly-His tagged proteins. Lanes 1 and 8 are Sigma low molecular weight markers (Product Code: M 3913). Lane 2 is a crude cell extract prepared with CelLytic-B™ that contains GST-GRB2 fusion protein. Lane 3 is the GST-GRB2 fusion protein after affinity purification from the CelLytic-B™ crude extract. Lanes 4 and 5 contain crude cell extract prepared with CelLytic-B™ that contains a bacterial alkaline phosphatase (BAP) with an N-terminal FLAG tag and a C-terminal 6-His tag. Lane 6 contains the BAP fusion protein that has been purified from the CelLytic-B™ crude extract using the ANTI-FLAG® M2 agarose gel (Product Code: A 1205) and eluted with FLAG peptide. Lane 7 contains the purified BAP fusion protein that has been purified from the CelLytic-B™ crude extract using a nickel chelate resin.*

The CelLytic-B™ extraction reagents can also be used with protease inhibitors if the recombinant protein is sensitive to degradation. These inhibitor cocktails, such as our formulations designed for general use (Product Code: P 2714), bacterial cell extracts (Product Code: P 8465) and poly(Histidine)-tagged proteins (Product Code: P 8849) are compatible with these reagents.

Conclusions

CelLytic-B™ and CelLytic-B™ II provide fast, effective extraction of soluble recombinant proteins. They do not interfere with the down-stream affinity purification of many commonly used recombinant tags.

References

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2. Hochstadt, J., Adenosine phosphoribosyltransferase from *Escherichia coli*, Meth. Enzymol., **51**, 558-567 (1978).

3. Schramm, V.L. and Leung, H.B., Adenosine monophosphate nucleosidase from *Azotobacter vinelandii* and *Escherichia coli*. Meth. Ezymol., **51**, 263-271 (1978).

About the Author

Rick Mehigh, Ph. D., is a principal scientist in Protein R&D at Sigma-Aldrich Corporation, St Louis, MO.

ORDERING INFORMATION

Product Code	Product Name	Unit	Price
B 3553	CelLytic-B™ Cell Lysis/Extraction Reagent	500 ml	$183.95
B 3678	CelLytic-B™ II Cell Lysis/Extraction Reagent	250 ml	$183.95

For more information, check CelLytic-B™ on the enclosed business reply card.
Life Science Catalog 2000-2001, page 78.