Whole Bacterial Culture Lysis for Affinity Purification of Histidine-tagged Proteins

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Abstract

The purification of recombinant proteins is a complex process involving multiple sample manipulations with the potential for protein loss at various steps. Traditional *E. coli* cell lysis methods require harvesting of cells that are then subjected to mechanical, detergent or enzymatic lysis to solubilize target proteins. The crude lysates must be clarified to remove cellular debris before application to an affinity resin. These time-consuming steps often lead to lower recovery of purified target proteins. CelLytic™ Express is a powder blend used to extract proteins from bacterial cells by lysis directly in the culture media. The lysis is complete in 15 minutes, leaving a clear protein solution, free of cellular debris. The lysis solution is applied directly to an affinity column, such as the HIS-Select™ Nickel or Cobalt Affinity Gel, for purification. Purification of affinity-tagged proteins using CelLytic Express routinely results in two to three times greater protein recovery compared to traditional lysis methods, without compromising purity. The method is optimized for use with histidine-tagged proteins but is applicable to other recombinant protein tags, such as FLAG®. A time-saving method for bacterial culture lysis was developed that eliminates the need for cell harvest or clarification of lysates prior to purification. Direct affinity absorption and purification of proteins from a whole bacterial culture will be demonstrated.

Introduction

The purification of recombinant proteins from *E. coli* hosts is a time-consuming, labor-intensive process which presents a number of challenges. Traditional lysis methods require the initial harvesting of the cells from the culture by centrifugation. Additionally, the collected cell paste is often subjected to multiple freeze/thaw cycles to facilitate cell lysis. Following cell lysis, the sample contains a large amount of cellular debris which must be removed by filtration or centrifugation prior to affinity purification. CelLytic Express eliminates the need for cell harvest or clarification steps. This unique lysis powder is added directly to the culture, resulting in a clear lysis solution. Using CelLytic Express, whole bacterial culture lysates can be applied directly to an affinity purification column. This method results in significant time savings, as well as increased protein recovery.

Methods

BL21 *E. coli* expressing a variety of recombinant proteins were grown in Terrific Broth and induced with IPTG using standard methods.

CelLytic Express lysis: CelLytic Express was added directly to the bacterial culture at a ratio of 50 mg per ml. The culture was briefly mixed to dissolve the lysis reagents. After incubation at room temperature for 15 minutes, the culture was visibly clear and ready to be applied to the affinity column.

Traditional cell lysis: Cells were harvested from the culture by centrifugation and the cell paste was subjected to one freeze/thaw cycle. For detergent lysis, a commercially available lysis reagent was added to the frozen cell paste at a ratio of 10 ml per gram. Enzymatic lysis buffer was prepared by adding Triton X-100 (0.1%) and lysozyme (0.2 mg/ml) to column buffer (300 mM NaCl in 50 mM $NaHPO_4$, pH 8). The cell paste was briefly vortexed to make a uniform suspension, then incubated at room temperature for 15 minutes with shaking. For sonication, frozen cell paste was suspended in column buffer at a ratio of 10 ml per gram. The suspension was sonicated with an ultrasonic probe using three 15-second bursts. Cellular debris was removed by centrifugation and the clear supernatant fluid was collected.

CelLytic Express inclusion body isolation: CelLytic Express was added directly to the culture containing inclusion bodies at a ratio of 50 mg per ml. The culture was incubated at room temperature for 15 minutes to lyse. The culture did not become clear. Inclusion bodies were collected by centrifugation and washed once with CelLytic Express dissolved in water. Alternatively, Celite filter aid was added to the lysis solution and the suspension was filtered through a bed of Celite. Guanidine hydrochloride, 8 M solution, was added to dissolve the inclusion bodies. The Celite was then removed by filtration.

Detergent lysis and isolation of inclusion bodies: The cells were collected from the culture. Detergent lysis reagent was added at a ratio of 10 ml per gram of cell paste, and the suspension was incubated at room temperature for 15 minutes with mixing. The inclusion bodies and cellular debris were collected by centrifugation. The solids were washed three times with detergent lysis reagent containing lysozyme and endonuclease to remove cellular debris and DNA. The inclusion bodies were dissolved in 8 M guanidine hydrochloride.

HIS-Select Nickel Affinity Gel purification: The prepared lysates were applied to HIS-Select Nickel Affinity Gel for IMAC (immobilized metal affinity chromatography) purification. The gel was packed into a column and equilibrated in column buffer. The column was washed with 5 mM imidazole in column buffer to remove loosely bound proteins. The proteins were then eluted with 250 mM imidazole in column buffer.

ANTI-FLAG® M2 Affinity Gel purification: The ANTI-FLAG M2 affinity gel was equilibrated in 0.01 M phosphate buffered saline, pH 7.4. Five ml of a BL21 *E. coli* culture expressing FLAG-GST was lysed with 50 mg per ml of CelLytic Express and purified using 1 ml of ANTI-FLAG M2 Affinity Gel. The loosely bound proteins were then washed from the gel using PBS, pH 7.4. The purified protein was then eluted from the resin with 0.1 M glycine, pH 3.0, and immediately neutralized.

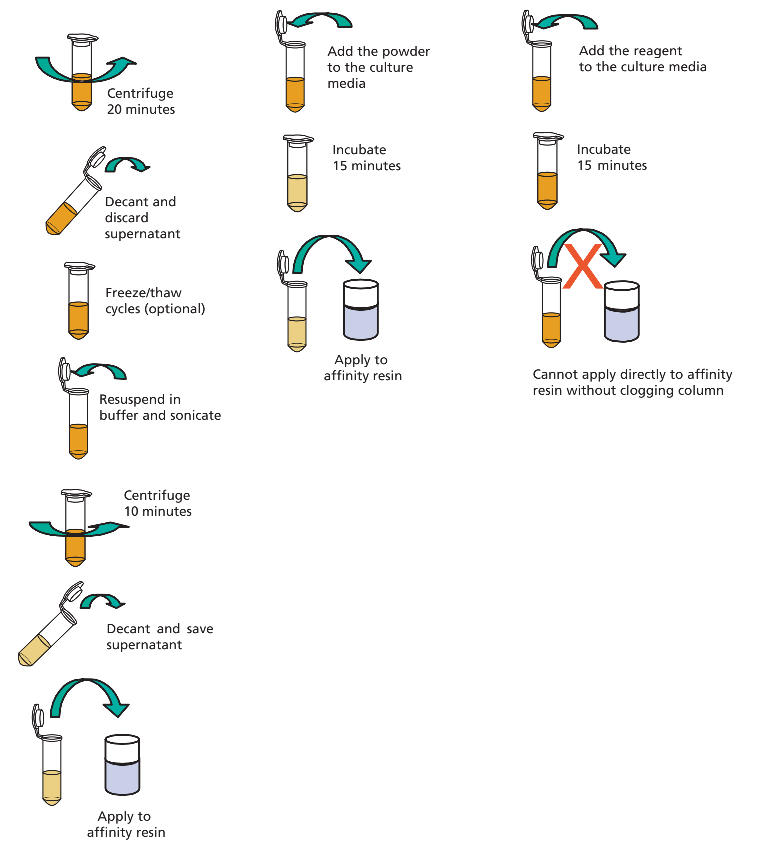

Figure 1. Traditional lysis methods which include harvesting cells from culture media and freeze/thaw cycles can take up to a day before the sample is ready for purification. Using CelLytic Express, the time is reduced to 15 minutes or less. Other commercially available reagents for whole culture lysis do not result in a clear solution.

Materials

- CelLytic Express (Product Code C 1990)
- HIS-Select Nickel Affinity Gel (Product Code P 6611)
- EZBlue™ Gel Staining Solution (Product Code G 1041)
- ANTI-FLAG M2 Affinity Gel (Product Code A 2220)
- Terrific Broth (Product Code T 9179)
- Bradford Reagent (Product Code B 6916)
- IPTG (Isopropyl β-D-1-thiogalactopyranoside, Product Code I 6758)
- Lysozyme (Product Code L 3790)
- Benzonase® Nuclease (Product Code E 1014)
- 8 M Guanidine Hydrochloride (Product Code G 9284)
- SigmaMarker™ Wide Range MW Marker (Product Code M 4038)

Results

In-culture lysis of *E. coli* cells

Figure 2. BL21 *E. coli* cells were grown to an optical density (OD_{600}) of 5.8. In-culture lysis reagent was added to each. CelLytic Express was added at a ratio of 50 mg per ml of culture. The other reagents were added according to the manufacturers' protocols. All three tubes were incubated at room temperature for 15 minutes with gentle agitation. CelLytic Express, left. Other culture lysis reagents, center and right.

CelLytic Express lysis resulted in a clear solution, completely free of any insoluble material. The other solutions were visibly murky and contained unsolubilized cellular debris.

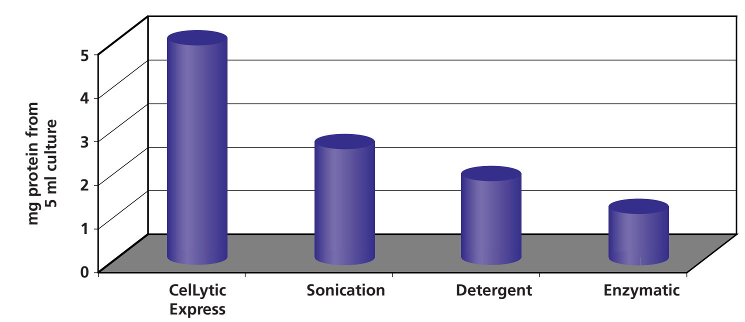

Figure 3. Target protein recovered from 5 ml of bacterial culture determined by Bradford assay.

Four samples of culture expressing p27-MAT, a metal affinity tagged protein, were lysed using various methods. Each lysate was affinity purified on 0.5 ml of HIS-Select Nickel Affinity Gel.

The purity of these four samples is exhibited in Figure 4.

With CelLytic Express, 2 to 4 times more protein was recovered than with the other lysis methods.

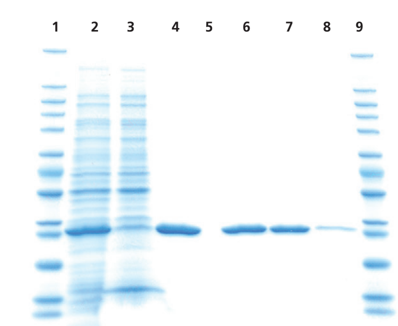

Figure 4. SDS-PAGE stained with EZBlue Gel Staining Reagent showing purity of protein from affinity purification using a variety of lysis methods.

A 27 kDa protein with a metal-affinity-tag, p27-MAT, was expressed in BL21 *E. coli* and lysed using four methods. The lysates were purified using IMAC on HIS-Select Nickel Affinity Gel.

Whole lysed culture (lane 2). Unbound protein (lane 3). Protein bound and eluted using CelLytic Express (lane 4). Empty (lane 5). Protein bound and eluted using sonication (lane 6), detergent lysis (lane 7) and enzymatic lysis (lane 8). Wide Range MW Markers, (lanes 1 and 9)

CelLytic Express lysis allows for greater protein recovery without sacrificing purity.

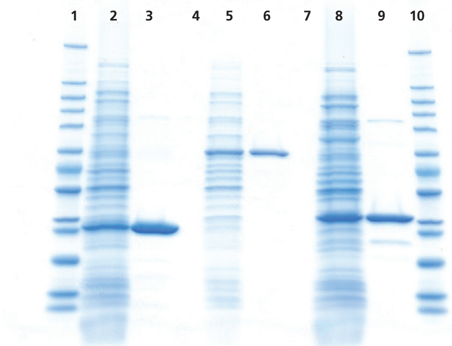

Figure 5. SDS-PAGE showing a selection of MAT-tagged proteins purified by HIS-Select chromatography following lysis with CelLytic Express.

Cultures expressing three different MAT proteins were lysed with CelLytic Express and purified on HIS-Select Nickel Affinity Gel. Lysed culture and elutions are shown for the purification of p27-MAT (lanes 2 and 3), FLAG-BAP-MAT (lanes 5 and 6) and FLAG-GrpE-MAT (lanes 8 and 9), respectively. Wide Range MW Markers (lanes 1 and 10).

Affinity purification using CelLytic Express has been compatible with every protein tested.

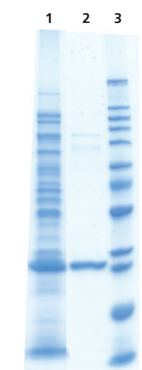

Figure 6. Purification of a FLAG-tagged protein.

FLAG-GST was expressed in BL21 *E. coli*. The culture was lysed with CelLytic Express and purified on ANTI-FLAG M2 Affinity Gel. Lysed culture and elution (lanes 1 and 2), respectively. Wide Range MW Marker (lane 3).

CelLytic Express, optimized for purification with HIS-Select Affinity Gel, also works well with other affinity purification systems.

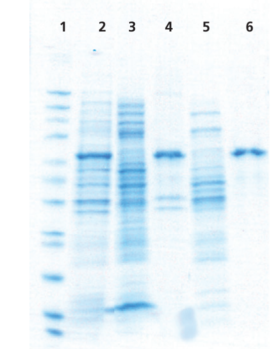

Figure 7. T4-DNA ligase, expressed as inclusion bodies, was recovered from *E. coli* lysates using both CelLytic Express and detergent lysis. Detergent lysis required additional enzymatic washes. The fact that the culture maintained its turbidity after lysis with CelLytic Express is an indication that inclusion bodies were formed. Whole culture (lane 2). Filtrate and inclusion bodies isolated using CelLytic Express (lanes 3 and 4), respectively. Filtrate and inclusion bodies isolated using detergent and enzymatic washes (lanes 5 and 6), respectively.

CelLytic Express allows for rapid detection and isolation of inclusion bodies. Sample manipulations are minimized, reducing handling time by 50% when compared to traditional methods.

Conclusions

- Fast, efficient whole bacterial culture lysis for protein purification
- Significant time saved by eliminating cell harvesting and clarification steps
- No need for extra equipment such as a sonicator or centrifuge
- Clear lysates ready to apply directly to affinity columns
- Higher protein yield without sacrificing purity
- Useful with a variety of affinity tag systems
- Rapid inclusion body detection and isolation

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