proteomics

CelLytic™ Express: Whole Bacterial Culture Lysis for Affinity Purification

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Application Notes

- Higher recombinant protein yields using whole culture lysis
- Saves time by eliminating cell harvest and clarification steps
- All-in-one formulation is optimized for protein extraction without additional reagents
- Compatible with IMAC, FLAG, and other affinity purification systems

Introduction

The expression of recombinant genes in *Escherichia coli* is a commonly employed method for quick and inexpensive production of target proteins. With the evolution of highly specific affinity purification techniques, protein isolation can often be accomplished with a single chromatographic separation. Traditional methods used for protein extraction involve multiple sample manipulations, which are often tedious and time-consuming. Typical *E. coli* cell lysis methods require an initial harvesting of the cells, which are then subjected to mechnical, detergent, or enzymatic lysis to release target proteins. Before application to an affinity resin, the crude lysates must be clarified to remove remaining cellular debris. These methods not only require time-consuming handling steps, but often lead to lower recovery of purified target proteins.

Sigma-Aldrich has developed CelLytic Express (Product Code C 1990), a non-denaturing and highly efficient protein extraction reagent, designed for bacterial cell lysis directly in the culture medium, which eliminates the need for cell harvest or clarification steps.

Highly efficient protein extraction and purification

Figure 1 illustrates a traditional extraction and purification procedure, compared with a method using CelLytic Express for lysis. Using CelLytic Express, sample handling is minimized, and the procedure time is reduced by at least 25%.

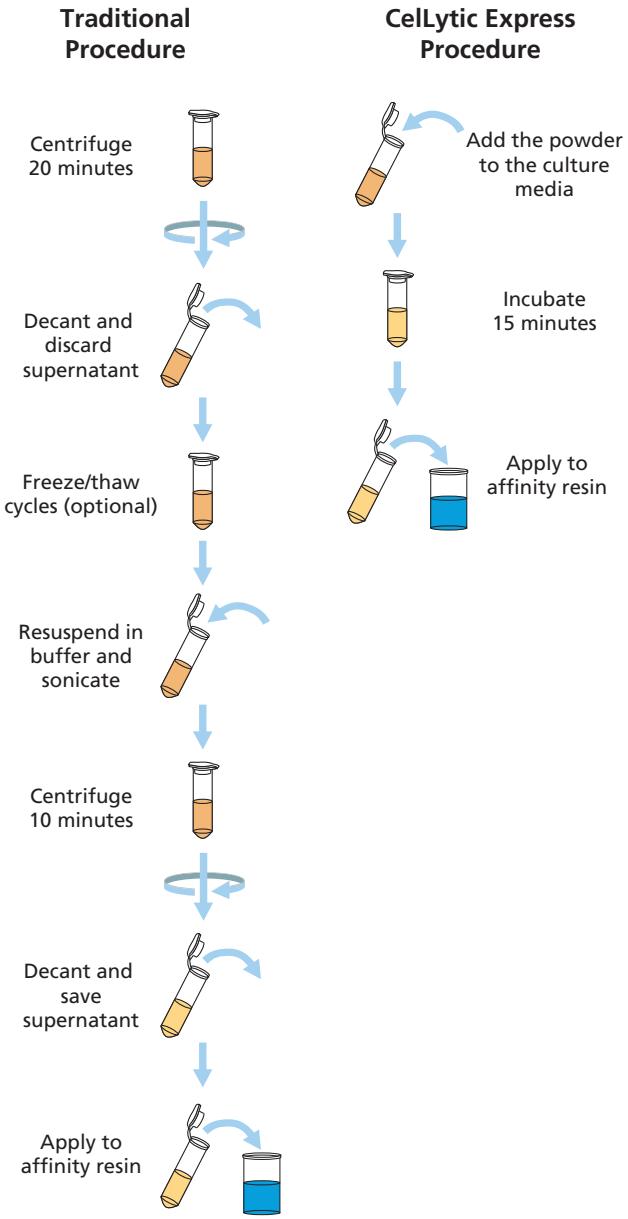


Figure 1. Comparison of a traditional affinity purification procedure with CelLytic Express lysis and purification. *CelLytic Express whole culture lysis simplifies affinity purification procedures and reduces procedure time by at least 25%.*

In order to demonstrate the utility of CelLytic Express, BL21 *E. coli* cells containing a plasmid coding for a 27-kDa metal affinity-tagged (MAT) protein were grown at 37 °C for 3 hours in sterilized Terrific Broth medium, supplemented with 100 µg/ml ampicillin. The cells were induced for production of the recombinant protein by the addition of IPTG (isopropyl-β-D-thiogalactopyranoside) to a concentration of 1 mM. The induced cells were incubated for an additional 3 hours at 37 °C. Four 10-ml aliquots of the bacterial culture were removed. Three cell samples were harvested by centrifugation at 2,000 x g for 20 minutes, and the spent cell media discarded. The first pellet was resuspended in 2.0 ml of column buffer (300 mM NaCl, 50 mM sodium phosphate, pH 8.0) and sonicated on ice, using four 15-second bursts. The second pellet was resuspended in 2.0 ml of 0.5% Triton® X-100, supplemented with 0.2 mg/ml lysozyme and incubated for 15 minutes at room temperature, with gentle mixing. The third cell pellet was lysed using the procedure for a commercially available detergent lysis solution. Each lysed cell sample was clarified by centrifugation at 14,000 x g for 10 minutes. The unharvested cell sample was lysed by adding 0.5 grams of CelLytic Express and incubating for 15 minutes at room temperature. All cell extracts were loaded onto 500-µl aliquots of pre-equilibrated HIS-Select Nickel Affinity Gel (Product Code P 6611). Following the loading step, the resin samples were washed and eluted using standard procedures. Total protein in each elution was quantitated using Bradford Reagent (Product Code B 6916) and is shown in Figure 2. Using CelLytic Express lysis, protein yield was at least twice as much as obtained with any of the standard methods tested. Samples of the eluted proteins were analyzed by SDS-PAGE as shown in Figure 3. CelLytic Express increases target protein yield, without sacrificing purity.

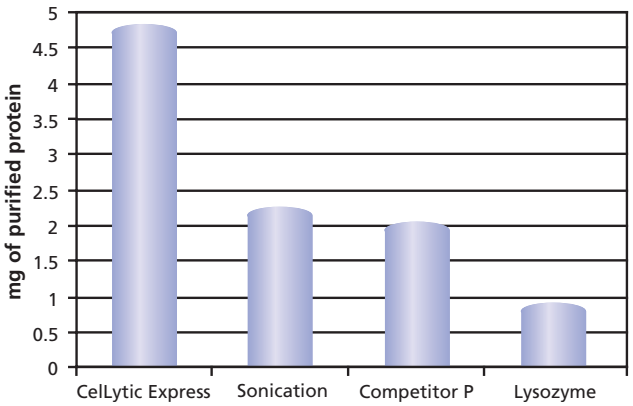
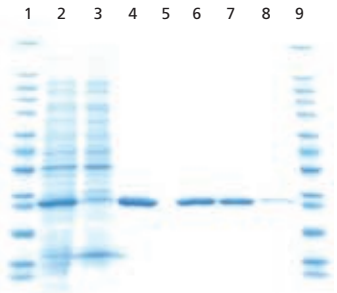


Figure 2. Comparison of total target protein recovered following various extraction methods and affinity purification. *Total protein eluted following each lysis method was quantitated using the Bradford protein assay.*



Lane 1 – Wide Range MW Marker (Product Code M 4038)
Lane 2 – CelLytic Express Whole Culture Lysate
Lane 3 – Unbound Fraction from CelLytic Express Purification
Lane 4 – CelLytic Express Elution
Lane 5 – Empty
Lane 6 – Sonication Elution
Lane 7 – Competitor P Elution
Lane 8 – Lysozyme Elution
Lane 9 – Wide Range MW Marker

Figure 3. SDS-PAGE analysis of purified protein samples. *Aliquots of the eluted protein (10 µl) obtained following each lysis method were mixed with an equal volume of Laemmli sample buffer (Product Code S 3401), and heated at 100 °C for 5 minutes. The proteins were separated on a 4-20% Tris-Glycine gel and stained with EZBlue™ Gel Staining Reagent (Product Code G 1041).*

Summary

CelLytic Express, a novel in-media lysis reagent, allows for rapid, efficient extraction and purification of recombinant proteins from bacterial cells. The non-denaturing lysis is complete in 15 minutes, resulting in a clear protein solution, free of cellular debris. Compared with traditional methods, using CelLytic Express for bacterial cell lysis increases protein yield, while reducing sample handling and procedure time.

Ordering Information

Product	Description	Unit
C 1990	CelLytic Express	10 x 25 ml 6 x 500 ml

Convenient package sizes that are pre-weighed powder suitable for indicated culture volumes.