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Rapid and Efficient Recovery of Histidine-Tagged Proteins Directly From Bacterial Cultures

SIGMA-ALDRICH

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Abstract

The purification of recombinant proteins is often a time-consuming and tedious process. Traditional protein purification methods from *E. coli* require an initial harvesting of cells, which are then subjected to mechanical, detergent, or enzymatic lysis to solubilize cellular protein(s). The crude cell lysates must then be clarified by centrifugation before applying to an affinity resin for purification. In order to overcome this bottleneck in proteomics research, Sigma-Aldrich has developed the HIS-Select™ iLAP™ (Integrated Lysis and Affinity Purification) 5 mL Column. The HIS-Select iLAP 5 mL Column is a single-use, disposable column designed for one-step purification of histidine-tagged proteins directly from a 5 mL bacterial culture. By eliminating the cell harvest and sample clarification steps, the iLAP technology streamlines the entire purification process, allowing for rapid recovery of the target protein(s). When compared to traditional methods, this improved procedure yields similar results and requires far less time and effort. Additionally, the purified protein yielded using the iLAP technology is suitable for a variety of downstream applications. In the present work, we have utilized the HIS-Select iLAP columns for the purification of a variety of Metal Affinity Tag (MAT) proteins and demonstrated the utility of the HIS-Select iLAP 5 mL Column for screening the expression level of a variety of bacterial clones expressing MAT-tagged proteins.

Introduction

The purification of recombinant proteins is a complex process involving multiple sample manipulations with the potential for protein loss at various steps. Traditional methods require the use of multiple reagents and equipment, such as a sonicator or french press, to purify recombinant proteins from bacterial cells. These methods lack throughput and require a great deal of hands-on work. Sigma-Aldrich has developed the HIS-Select iLAP 5 mL Column, a single-use, disposable column designed for the one-step purification of histidine-tagged proteins directly from bacterial cultures. Using the HIS-Select iLAP Column 5 mL Column, purification time is reduced by 65%, without sacrificing the purity or yield of the final product.

Materials

All products were obtained from Sigma-Aldrich (St. Louis, MO), unless otherwise noted.

- **HIS-Select iLAP 5 mL Column** (Cat. No. H9913)
- **HIS-Select Wash & Elution Buffer Kit** (Cat. No. HS0100)
- **HIS-Select Nickel Affinity Gel** (Cat. No. P6611)
- **CelLytic™ B Cell Lysis Reagent** (Cat. No. B7435)
- **Terrific Broth, Modified EZMix™ Powder** (Cat. No. T9179)
- **BL21 Competent Cells, Uni-Pack (DE3) pLysS-T1R** (Cat. No. B3310)
- **Isopropyl-β-D-1-thiogalactopyranoside** (Cat. No. I6758)
- **Ampicillin, 100 mg/mL, 0.22 μm filtered** (Cat. No. A5354)
- **Bradford Reagent** (Cat. No. B6916)
- **EZBlue™ Gel Staining Reagent** (Cat. No. G1041)
- **Sample Buffer, Laemmli 2× Concentration** (Cat. No. S3401)

Methods

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

HIS-Select iLAP 5 mL Column Purification

5 mL of bacterial culture expressing a histidine-tagged recombinant protein was added to the column, and the top sealed. The column was briefly vortexed and mixed for 15–20 minutes to allow for lysis of the cells and solubilization of the cellular debris. The column was drained, allowing the resin to collect on the frit. The column was washed with HIS-Select Wash Buffer, (300 mM NaCl, 50 mM sodium phosphate, 10 mM imidazole) diluted 2× in water, to remove loosely bound proteins. The remaining protein was eluted from the column using HIS-Select Elution Buffer (300 mM NaCl, 50 mM sodium phosphate, 250 mM imidazole).

Traditional Purification

5 mL of bacterial culture expressing a histidine-tagged recombinant protein was centrifuged for 20 minutes at 2000 x *g* to harvest the cells. The spent cell media was decanted off, and the pellet resuspended in 2 mL of CelLytic B (Sigma method) or 2 mL of column buffer (Competitor Q). The Sigma sample was incubated for 10 minutes at room temperature. The Competitor Q sample was sonicated three times for 15 seconds. Each sample was centrifuged for 12 minutes at 15,000 x *g* to pellet any insoluble cellular debris. The supernatant was loaded onto each affinity resin for purification.

GST-MAT Dilution Experiment

A series of samples was prepared by diluting a BL21 *E. coli* culture expressing metal affinity- tagged glutathione-S-transferase. Each sample contained either 1, 2, 3, 4, or 5 mL of the original culture, and the final volume was brought to 5 mL with sterile Terrific Broth. This set of samples was purified using HIS-Select iLAP 5 mL Columns, and the eluted protein quantitated by Bradford assay.

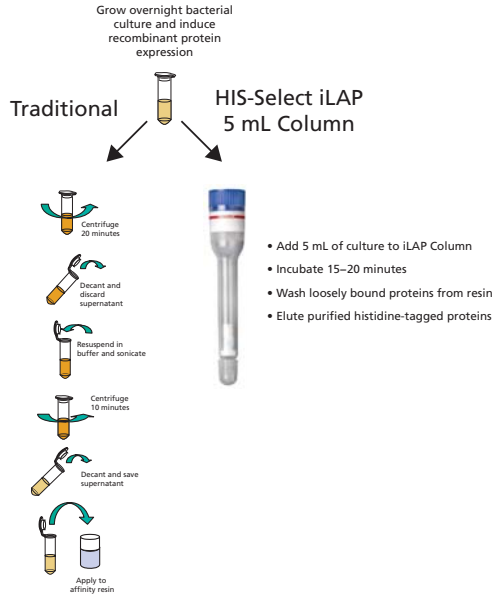

Figure 1: Comparison of iLAP Purification With Traditional Methods. By using the HIS-Select iLAP 5 mL Column, the number of sample handling steps is greatly reduced, resulting in significant time savings. Additionally, the procedure does not require the purchase of additional reagents or equipment.

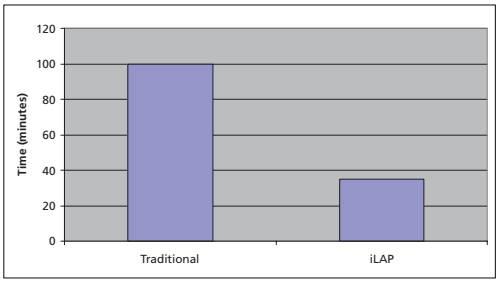

Figure 2: Time Savings Using the iLAP Purification Method. By utilizing the iLAP technology, the time required for protein purification is reduced by 65% compared against the traditional workflow, without sacrificing yield or purity of the final product.

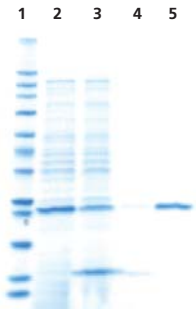

Figure 3: Histidine-Tagged Protein Purification Using the HIS-Select iLAP 5 mL Column. A BL21 *E. coli* culture expressing GST-MAT (lane 2) was prepared as described in the Methods section. By utilizing the iLAP Column, the metal affinity-tagged protein was purified (lane 5) directly from the bacterial culture in approximately 35 minutes. The iLAP method saves time without sacrificing purity. Lanes 3 and 4 contain the column flow-through and wash, respectively.

Results

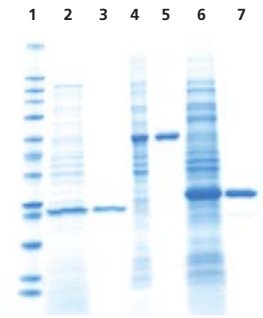

Figure 4: Purification of a Variety of Metal Affinity-Tagged Proteins Using the HIS-Select iLAP 5 mL Column. BL21 *E. coli* cultures expressing GST-MAT, FLAG-BAP-MAT, and GrpE-MAT (lanes 2, 4, and 6, respectively) were applied to a HIS-Select iLAP 5 mL Column for purification. The target protein (lanes 3, 5, and 7) was recovered with exceptional purity in approximately 35 minutes. The HIS-Select iLAP 5 mL Column can be used for the purification of a variety of histidine-tagged proteins without sacrificing purity.

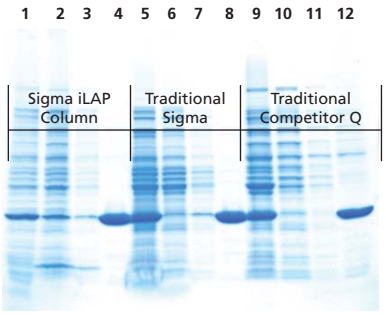

Figure 5: Comparison of Histidine-Tagged Protein Purification Methods. Histidine-tagged protein purification using the HIS-Select iLAP 5 mL Column (lanes 1–4) was compared with traditional methods [using both Sigma (lanes 5–8) and competitor Q (lanes 9–12) protocols]. The SDS-PAGE gel above depicts the column load, flow-through, wash, and elution fractions for each purification method. 5 mL BL21 *E. coli* cultures were purified using "traditional" methods or the HIS-Select iLAP 5 mL Column (as outlined in Methods). Although the HIS-Select iLAP 5 mL Column required 65% less time, the purity of the final eluted protein is as good, or better, than the traditional methods. A significant amount of non-specific protein was found in the Competitor Q elution fraction (lane 12).

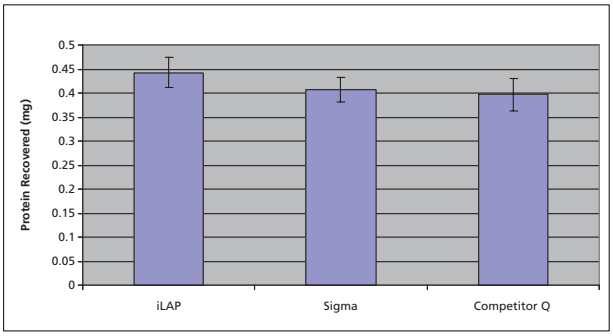

Figure 6: Purified GST-MAT Eluted from Column Using Various Protein Purification Procedures. A 5 mL BL21 *E. coli* culture expressing GST-MAT was purified using three procedures. The purification procedure using the iLAP column was completed in an average of 35 minutes. The standard purification procedures (using Sigma and Competitor Q reagents) averaged over 100 minutes. The total amount of protein eluted from the column using all three methods was comparable. Total protein was determined using Bradford assay.

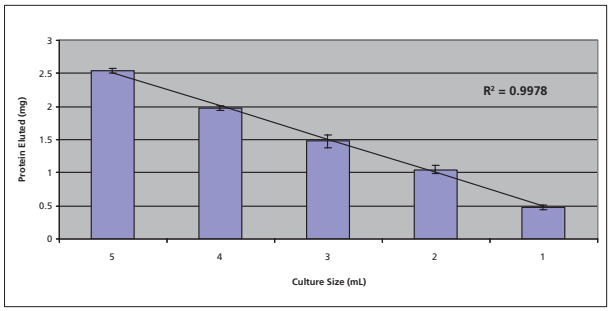

Figure 7: Purification of GST-MAT Dilution Series. A BL21 *E. coli* culture expressing GST-MAT was prepared in a dilution series as described in the Methods section. Using the HIS-Select iLAP 5 mL Column, the metal affinity-tagged protein was purified directly from the bacterial culture in approximately 35 minutes. The target protein was quantitatively recovered from the bacterial culture, demonstrating the utility of applying the HIS-Select iLAP 5 mL Column for screening the expression level of multiple clones expressing histidine-tagged recombinant proteins. The R^2 value of 0.9978 indicates excellent linearity of fit for data points along the GST-MAT dilution series. Total protein eluted was determined using the Bradford assay.

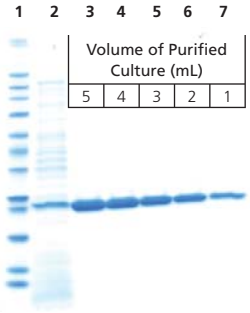

Figure 8: Purification of GST-MAT Dilution Series. A BL21 *E. coli* culture expressing GST-MAT (lane 2) was prepared in a dilution series as described in the Methods section. Using the HIS-Select iLAP 5 mL Column, the metal affinity-tagged protein was purified directly from the bacterial cultures in approximately 35 minutes. The recovery of histidine-tagged protein is directly proportional to the volume of culture applied to the column (see **Figure 6**). The protein eluted from the column is highly pure, even at sub-saturating levels (lanes 5–7).

Conclusions

- The iLAP technology yields purified histidine-tagged protein in as little as 30 minutes
- All-in-one iLAP formulation eliminates the need for additional reagents and equipment
- Gentle, non-denaturing lysis maintains protein activity
- The iLAP platform is useful for screening expression of histidine-tagged proteins in multiple clones

References

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2. Porter, J. J.; Mehigh, R. J.; Kappel, W. K.; Scott, G. B. I. CelLytic Express: Whole Bacterial Culture Lysis for Affinity Purification. *Sigma-Aldrich Life Science Quarterly* **2003**, *5*, 18–19.

3. Kappel, W. K.; Mehigh, R. J.; Jenkins, E. A. U.S. Patent application 20,040,259,162, filed on May 3, 2004.