Rapid His-Tag Purification of Recombinant Proteins Using Dionex ProPac IMAC Columns

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Introduction

Immobilized-metal-affinity chromatography (IMAC) stationary phases are typically used for the capture/release enrichment of proteins in the purification of His-tag recombinant proteins. The IMAC approach captures His-tagged proteins, which have an affinity for the immobilized metal bound to the IMAC column. Release of the bound His-tagged protein is achieved by exposure to an elution buffer containing a high concentration of imidazole. Typical materials used for this purification are medium-pressure, porous resins such as agarose or cross-linked dextran containing a chelation function, which can be charged with the appropriate metal for the purification. From 500 to 1000 mL cultures, 5 to over 50 mg of protein can be obtained depending on the expression and fermentation process. The columns used at this level of protein production are usually 16 × 200 mm (30 mL total volume) with a flow rate of around 5 mL/min.

A typical purification protocol would include the following steps:

1. Wash with EDTA and 30 mL water; then, add 30 mL of 100 mM EDTA, followed by 30 mL water to obtain a total volume of 90 mL
2. Charge with 30 mL of 100 mM nickel sulfate ($NiSO_4$)
3. Equilibrate with 100 mL loading buffer
4. Load with sample
5. Wash the column with 200 mL loading buffer
6. Elute with imidazole

The process outlined here takes up to 3 h and will release the protein in around 50 mL of buffer containing high concentrations of imidazole and sodium chloride. As a result, further concentration and buffer-exchange steps must be performed, which are quite lengthy processes, to get the protein into a more manageable volume and buffer conditions.

The Dionex ProPac® IMAC-10 column is an HPLC resin column using 10 µm pelicular polystyrene beads with isolated poly(IDA) grafts on a hydrophilic boundary layer. The capacity of a 4 × 250 mm column is compatible with the levels of protein expected from 1 L cultures. For higher levels, 9 and 22 mm columns are available.

As the ProPac column is a pressure-stable column, flow rates of 2 mL/min are easily achieved during the washing stages and the low-dead-volume of the column (around 1 mL due to the pellicular structure) allows many column volumes to be used for washing, and reduced flow to be used during elution to increase the concentration of the purified protein.

Equipment

UltiMate® 3000 Titanium system consisting of:

- Analytical titanium pump
- Thermal compartment with two column change valves
- VWD detector

WPS-3000 Biocompatible autosampler with fractionation, 250 µL syringe, 1 mL loop

ISCO Foxy® Jr. Fraction collector

Chromeleon® Chromatography Data System software with fractionation license

Dionex ProPac IMAC-10, 4.0 mm i.d. × 250 mm

Agilent Zorbax® GF-250, 4 µm, 4.6 × 250 mm

Preparation of Samples

Using genomic DNA from *S. staphylolyticus* as a template, the mature lysostaphin ORF from the lysostaphin precursor (glycyl-glycine endopeptidase) sequence (Gen Bank Accession No. X06121) was amplified using PCR. The gene was first cloned in pCR-Blunt® vector using the Zero Blunt Cloning system (Invitrogen Ltd, UK) and then subcloned in the pET vector cloning system using pET-28a vector (Invitrogen Ltd, UK).

Pel10Acm, a carbohydrate module encoded in the pel10A gene originating from *P. cellulosa* was amplified using PCR. The PCR product was cloned into pGEM®-T Easy vector (Promega, UK) and then subcloned in the pET vector cloning system using pET-28a vector (Invitrogen Ltd, UK).

Recombinant pET vector DNA encoding lysostaphin and Pel10Acm was transformed into *E. coli* BL21(DE3) for protein expression. The transformed *E. coli* BL21(DE3) was used to inoculate an LB starter culture which was incubated overnight at 37 °C and 200 rpm. The LB starter culture was then inoculated into a 1 L flask of LB media. The inoculated LB media was incubated at 37 °C and 200 rpm until an o.d.600 of 0.6–1.0 was obtained. At this point, protein expression was induced by addition of 0.24 µg/mL IPTG and incubation at 30 °C and 100 rpm. The cell-free extract was harvested through centrifugation of cells at 4000 x g for 15 min. The resulting cell pellet was resuspended in IMAC buffer A and sonicated at 14 mA every 10 s for a total of 2 min. The sonicated cells were centrifuged at 24,000 x g for 20 min and the resulting cell-free extract was applied to the nickel columns for purification of recombinant lysostaphin and Pel10Acm.

Methods

Washing and charging the column can be done with simple 1 mL loop injections. The loading of the column with concentrated cell lysate is done using line C of the quaternary titanium pump.

Chromatographic Conditions– UltiMate 3000 Titanium System

Column:	Dionex ProPac IMAC-10, 4.0 mm i.d. × 250 mm (P/N 063278)
Mobile Phase:	A) Sodium phosphate (20 mM), Sodium chloride (500 mM), Imidazole (20 mM), pH 7.4
	B) Sodium phosphate (20 mM), Sodium chloride (500 mM), Imidazole (500 mM), pH 7.4
Gradient:	See Table 1
Flow Rate:	See Table 1
Sample Volume:	66 mL (loading via line buffer line C)
Detection:	Absorbance at 280 nm
Column Preparation:	Column stripped and charged through loading of 500 µL EDTA (manual 500 µL injection), 1000 µL of nickel sulfate (manual 1000 µL injection)

The concentrated cell lysate of 33 mL, was pumped onto the column at 1 mL/min. Following sample loading, the column was washed with the loading buffer before elution with an imidazole gradient at 0.5 mL/min.

Table 1. Gradient Table

Time (min)	Flow (mL/min)	%B	%C
0.000	1.000	0.0	0.0
0.100	1.000	0.0	0.0
0.200	1.000	0.0	100.0
33.000	1.000	0.0	100.0
33.100	1.000	0.0	0.0
53.000	0.500	0.0	0.0
103.000	0.500	100.0	0.0
103.100	1.000	0.0	0.0
128.000	1.000	0.0	0.0

Results

The purification procedure takes approximately 2.15 h compared to 3 h using the previous media. The length of purification is dependent on sample volume and therefore, the method duration can be easily reduced to accommodate lower sample volumes. In addition to time efficiency, the ProPac IMAC column produced a more concentrated end product, which is important for postpurification sample processing. In this example, the protein was collected in a total volume of 8 mL, which was concentrated and desalted within a few hours. An IMAC purification was performed using a Chelating Sepharose™ Fast Flow, XK 16/20 column. The purification used the same amount of protein and was eluted in a total volume of 50 mL, which required concencentration over several days. The less time spent concentrating the purified protein dramatically reduces the total purification time. The elution profiles for the recombinant proteins Pel10Acm and lysostaphin are shown in Figures 1 and 2.

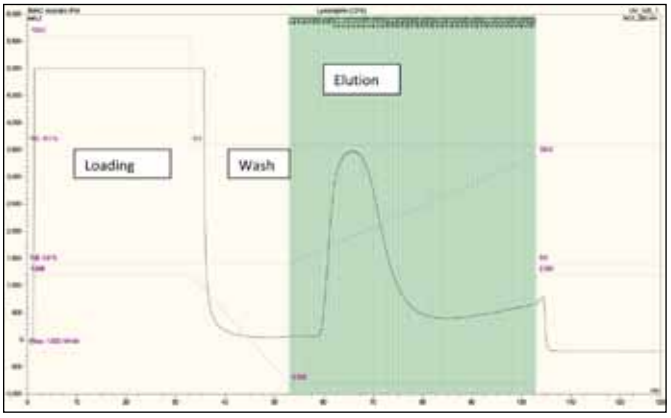

Figure 1. IMAC Purification of Pel10Acm from an E. coli *cell-free extract.*

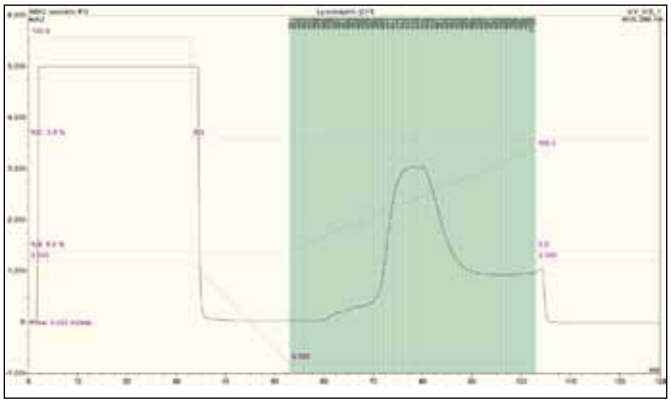

Figure 2. IMAC purification of lysostaphin from an E. coli *cell-free extract.*

The collected lysostaphin protein fractions contained 9.5 mg of the purified recombinant protein in an 8 mL elution buffer. A duplicate sample was applied to a Chelating Sepharose Fast Flow IMAC column and collected a similar amount of protein in a 50 mL elution buffer. The two methods produced similar purification results as shown by the amount of protein collected and the subsequent analysis by gel filtration (Figure 3).

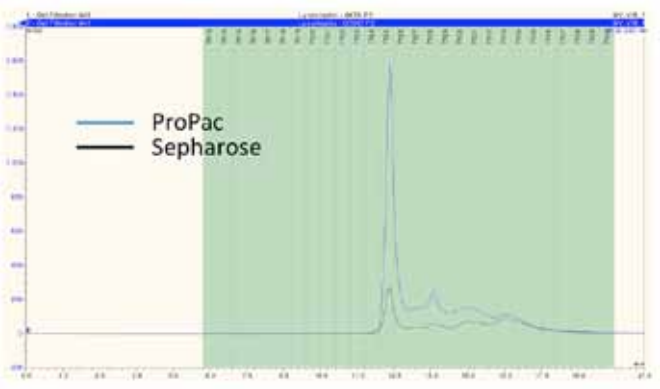

Figure 3. Gel filtration analysis of lysostaphin from ProPac and Sepharose IMAC columns. 50 mM PO_4, 100 mM NaCl, pH 7.0 with a flow rate of 0.25 mL/min, 30 µL of the collected fractions injected, UV detection at 280 nm.

Gel filtration analysis of the purified lysostaphin samples from the ProPac and the Sepharose columns are shown in Figure 3. The ProPac sample is more concentrated but both purified samples show a similar profile with no indication of aggregation.

Conclusion

The Dionex ProPac IMAC column can be used to successfully and rapidly purify His-Tagged recombinant proteins from cell-free extracts harvested from up to 1 L of *E. coli* fermentation cultures.

The ProPac IMAC column achieved the purification in 90 min compared with 3 h previously. Purification time may be reduced further through the application of a more concentrated sample with a smaller sample volume. The released protein was collected in 8 mL buffer, which is a more manageable volume compared to the 50 mL released from the Chelating Sepharose Fast Flow column. With larger collection volumes, a lengthy concentration step is required. Concentration of 50 mL using ultrafiltration spin columns may take several days. Dialysis to remove the high salt concentration will also require several buffer exchanges. The smaller collection volumes reduce the time required for these steps considerably.

This application note has been kindly provided by a Dionex customer, and in the opinion of Dionex represents an innovative application of Dionex products. This application has not been tested in a Dionex applications lab and therefore its performance is not guaranteed.

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No ISO data available for included figures.

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