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Purification of His-Tagged Proteins by Metal Affinity Chromatography with Sartobind® IDA Lab

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Abstract

One of the practical methods to isolate recombinant proteins is to label them for purification by affinity chromatography. A common protein tag for this purpose is a peptide of six to eight histidine residues (His_6 or His_8) cloned at the N- or C-terminus, which enables purification by metal chelate chromatography. This modality is based on the complexing of metal ions such as Ni^{2+}, Cu^{2+} or Co^{2+}, immobilized on a chromatography matrix, by chelate formation through the imidazole ring of the amino acid histidine. The bound His_6-tagged protein can subsequently be eluted from the matrix selectively with imidazole, which competitively binds to the metal complex. Sartobind® IDA utilizes a cellulosic membrane matrix, functionalized with iminodiacetic acid (IDA). This membrane therefore enables the immobilization of any suitable metal ion for metal affinity purification. In this study, Sartobind® IDA Lab units were evaluated for binding capacity, effectiveness, and handling in the purification of two hexa-histidine tagged proteins.

Find out more: www.sartorius.com/en/products/lab-filtration-purification/membrane-chromatography

Introduction

GFP is commonly used as a reporter protein. During the autocatalytic process, GFP forms a structure that emits green light at 508 nm if stimulated by light with wavelength of between 396 and 475 nm (absorbance maxima). Therefore, it is relatively easy to follow GFP during processing by means of fluorescence light. However, it becomes unstable or loses its fluorescent under certain conditions. GFP may be intracellular when produced in *E. coli* and therefore cells must be disrupted prior to separation.

The gene of β-glucanase is a fusion of glucanase genes from *Bacillus macerans* and *Bacillus amyloliquefaciens* [Borris *et al.,* 1990]. This fusion was cloned into a pET20b+ vector, while the signal sequence of β-glucanase was preserved. Transcription in *E. coli* was carried out under the IPTG-inducible T7 promoter. A secretion cassette is located on the plasmid. It consists of the gene for the bacterial release protein (kil) under the control of the weak stationary phase promoter fic [Miksch *et al.,* 1997]. The moderate expression of kil allows the secretion of the glucanase from the periplasm into the culture medium. The isolation and purification of β-glucanase can therefore be carried out after removal of *E. coli* from the fermentation medium.

Sartobind® IDA Lab offers the possibility to purify virtually any his-tagged target protein by affinity chromatography. Supplied uncharged, the user is free to choose the metal ion of their choice, to achieve optimum performance. Furthermore, the integral Luer connectors enable convenient use with syringes, while UNF 10-32 adapters included with the devices provide flexibility for operation by peristaltic pump or liquid chromatography systems. In this study, we demonstrate the performance of Sartobind® IDA Lab units (Figure 1) for metal affinity purification, using Cu^{2+} to isolate both GFP and β-glucanase carrying His_6 tags at their N-termini.

Figure 1: *Sartobind® IDA Lab Membrane Adsorber Unit*

Materials and Methods

Production of His_6-GFP
BL21 *E. coli* were transfected with pHis_6-GFP. Cells were cultivated in 300 mL medium (10 g/L peptone, 10 g/L yeast extract, 20 g/L glycerol, 10 g/L NaCl, 100 mg/L ampicillin) in a 1 L baffled flask, inoculated with 1 mL of starter culture. The culture was incubated with shaking at 37 °C overnight, with gene expression induced by IPTG after 2 hours. The cells were isolated by centrifugation, the resulting pellet (23.3 g) suspended in approximately 10 mL of lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 8.0) and 25 mg lysozyme dissolved in 1 mL buffer was added (final concentration = 1 mg/mL). Cells were disrupted in an ice bath using pulsed ultrasound and the resulting lysate centrifuged. After removal of 5 mL for analysis, the supernatant was passed through a 0.2 µm filter and the filtrate was taken forward for purification.

Production of His_6-β-glucanase (His_6-bgl)
BL21 (DE3) *E. coli* cells transfected with pET-bgl-His_6-sec were cultivated under the same conditions as for His_6-GFP production. The cells were harvested by centrifugation at 11,000 *g* for 30 min. A portion of the supernatant was passed through a 0.2 µm filter and the filtrate was used for purification.

Preparation of Sartobind® IDA Lab Unit
Each Sartobind® IDA Lab unit containing 9.3 cm^2 adsorption area (3x layers of 3.1 cm^2 membrane discs) was flushed with 10 mL of deionized water, which was slowly pumped from bottom to top until the unit was vented completely. Then the remaining water was applied to the top of the unit and allowed to flow through by gravity. The following solutions were passed through the unit in the same way:

1. 0.5 M $CuSO_4$ (charging buffer)
2. 10 mM imidazole in 25 mM potassium phosphate buffer, pH 8 (equilibration buffer 1)
3. 25 mM potassium phosphate buffer, pH 8 (equilibration buffer 2)

Protein Purification
The filtered lysate of His_6-GFP (11 mL) and cell-free supernatant of His_6-bgl (80 mL) were applied to the Sartobind® IDA Lab units and the flow through fractions collected. The units were washed twice with 10 mL of washing buffer (50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, pH 8.0). Both fractions were collected and pooled. The proteins were eluted by applying 2x 3 mL of elution buffer (50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole, pH 8.0). Each eluate fraction was retained separately and all samples were stored at -20 °C until analysis.

Protein Analysis
Lysate | cell-free samples, and flow through, wash and eluate fractions were analyzed by polyacrylamide gel electrophoresis (PAGE) to assess purity. Protein activity was also assessed by fluorescence estimation for GFP or enzymatic activity of β-glucanase.

Results and Discussion

His_6-GFP
Cell lysate (Figure 2, lanes 2 and 3) and Sartobind® IDA Lab flow through fraction (lane 4) show a broad range of intracellular proteins. Only the bands of abundant proteins are visible in the wash fractions (lane 5). Eluate 1 (lane 6) shows a clear band below 30 kDa, corresponding to His_6-GFP with an expected molecular weight of 27.7 kDa. The two other bands are intracellular proteins which were also bound by the membrane adsorber – a common occurrence in purifications from whole cell lysates. Eluate 2 (lane 7) showed no detectable proteins, indicating that all bound proteins were recovered in the first elution step.

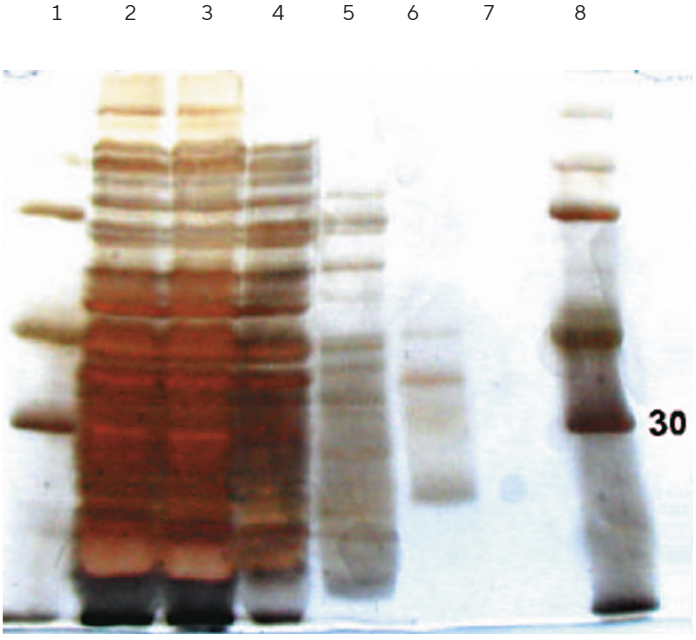

Figure 2: *Polyacrylamide Gel Analysis of His_6-GFP Purification Samples*

Lane 1: Molecular weight standards
Lane 2: Lysate
Lane 3: Filtered lysate
Lane 4: Flow through
Lane 5: Wash fractions (pooled)
Lane 6: Eluate 1
Lane 7: 7 Eluate 2
Lane 8: Molecular weight standards

His_6-β-glucanase
The samples of cell culture supernatant and Sartobind® IDA Lab flow through were concentrated prior to PAGE. These samples show a distinct, broad band migrating to <30 kDa (Figure 3, lanes 2-4), which corresponds to a high quantity of β-glucanase. The high quantity of this target protein in the flow through suggests that the amount of β-glucanase exceeded the binding capacity of the Sartobind® IDA Lab unit. The pooled wash fractions (lane 5) show no detectable protein, indicative of stable binding of the target molecule to the membrane adsorber. Each eluate (lanes 6 and 7) displays a single band of the target protein. This indicates that the membrane adsorber unit was completely saturated with the target protein, in keeping with the previously mentioned detection of a high quantity of β-glucanase in the flow through. No additional proteins were detected in the eluates.

The protein concentration in eluates 1 and 2 was 232 and 127 µg/mL, respectively, corresponding to a total protein content of 1,077 µg in the 2x 3 mL fractions. This places the binding capacity of Sartobind® IDA Lab for β-glucanase at approximately 1.1 mg, although we assume a third eluate would have yielded further protein and therefore this represents the minimum capacity of the unit (118 µg/cm^2 or 4.3 mg/mL, where 1 mL membrane = 36.4 cm^2).

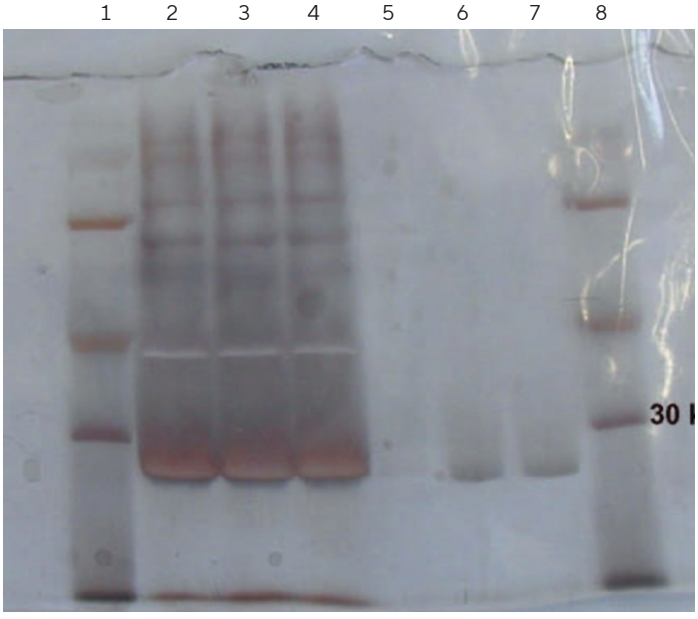

Figure 3: *Polyacrylamide Gel Analysis of His_6-bgl Purification Samples*

Lane 1: Molecular weight standards
Lane 2: Lysate
Lane 3: Filtered lysate
Lane 4: Flow through
Lane 5: Wash fractions (pooled)
Lane 6: Eluate 1
Lane 7: 7 Eluate 2
Lane 8: Molecular weight standards

Conclusion

Our results show that, when charged with Cu^{2+}, Sartobind® IDA Lab units are suitable for the purification of both intra- and extra-cellular proteins. The units exhibited high binding capacities for both His_6-GFP and His_6-β-glucanase, and both proteins were eluted at high purity.

References

Borris, R., Buettner, K. and Maentsaelae, P. (1990). Structure of the beta-1,3-1,4-glucanase gene of Bacillus macerans: homologies to other beta-glucanases. Molecular & general genetics 222 (2-3), 278-283.

Miksch, G., Fiedler, E., Dobrowolski, P., Fries, K. (1997). The kil gene of the ColE1 plasmid of Escherichia coli controlled by a growth-phase-dependent promoter mediates the secretion of a heterologous periplasmic protein during the stationary phase. Arch. Microbiol. 167 (2+3), 143-150.

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Note

Literature published up to c.2022 may reference the use of Sartobind® MA, which is a name previously used for the Sartobind® Lab membrane adsorbers. When these products were renamed, there was no change made to fit, form or function. Therefore, results collected and methods established using Sartobind® MA remain valid also for Sartobind® Lab.

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