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Scouting Protein Purification Conditions Using Vivapure® Centrifugal Ion Exchange Membrane Absorbers

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Abstract

Recombinant proteins are important tools for developing our understanding of protein structure and function, forming the basis for the development of novel drugs and biologics. For reliable results, these proteins must be prepared to high purity. In this study, a scouting procedure is described where optimal purification conditions for SH2 domain were developed using Vivapure® IEX centrifugal devices. In a first step, protein binding to two different membrane adsorbers was evaluated with several different buffer formulations. The optimum membrane and buffer combination were then further refined by adjusting the salt concentration used for elution of the target protein. The results demonstrate that centrifugal membrane adsorber devices offer the ideal solution for rapid determination of optimal ion exchange purification conditions, thanks to the ability to test multiple options in parallel with a simple bind-wash-elute procedure.

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

Introduction

For separation and purification of proteins from biological samples, different characteristics of the target protein e.g. its size, charge, hydrophobicity or specifically engineered tags, are exploited.

With ion exchange chromatography, separation is achieved on the basis of charge differences between biomolecules. This makes it a versatile method often used for pre-fractionation or purification of a target protein from crude protein mixtures. To optimize the purification procedure for an individual target, several binding and elution conditions have to be tested on cation and anion exchange matrices.

In contrast to traditional column chromatography methods, Vivapure® IEX centrifugal columns allow scouting of several chromatography conditions in parallel, leading quickly to different fractions which can be further analyzed for enriched or even already purified target protein.

Here, we demonstrate the performance of Vivapure® IEX Mini spin columns for evaluation of optimal purification conditions of cloned SH2 domains from an *E. coli* lysate in a two step procedure. This protocol can generally be employed for identifying a purification method based on ion exchange chromatography for a given target protein, as it is fast and only uses small amounts of the sample.

In the first step of this study, binding conditions were evaluated by loading the sample on Vivapure® Q and S columns at various pH values, eluting bound proteins with a high salt concentration buffer and analyzing all fractions for the target protein. The results from this experiment provided the optimal binding pH and the best ion exchange chemistry for the purification of SH2 domain.

In a second step, the best elution method was evaluated by applying increasing salt concentrations to columns which were shown to bind the target protein in step one, leading to a complete purification protocol in less than one hour.

Materials and Methods

Table 1

Buffers tested to determine the optimum pH and salt concentration for binding and elution in ion exchange purification of SH2 domain.

Buffer A	25 mM Citrate, pH 4
Buffer B	25 mM Potassium phosphate, pH 6
Buffer C	25 mM HEPES, pH 8
Buffer D	25 mM Sodium bicarbonate, pH 10
Buffer E	25 mM Citrate, pH 4, supplemented with 1 M NaCl.
Buffer F	25 mM Potassium phosphate, pH 6, supplemented with 0.2 M, 0.4 mM, 0.6 mM, 0.8 mM and 1 M NaCl, respectively.
Buffer G	25 mM HEPES, pH 8, supplemented with 1 M NaCl
Buffer H:	25 mM Sodium bicarbonate, pH 10, supplemented with 1 M NaCl

Scouting Binding Conditions

300 mL LB media was inoculated with 4 mL of an overnight culture and incubated at 37°C, shaking at 150 rpm until an OD600 of 1.0 was reached. IPTG was added to a final concentration of 1 mM and the culture incubated for a further 4 h with shaking at 150 rpm. Cells were harvested by centrifugation at 4,000 *g* for 30 min at 4°C. The pellet was resuspended in 35 mL PBS (150 mM KPi, pH 7.3) and cells were lyzed by addition of lysozyme to a final concentration of 0.1 mg/mL and incubation for 1 h at 37°C. Insoluble particles and cell debris were removed by centrifugation at 10,000 *g* for 30 min at 4°C.

4 x 200 µL aliquots of the cell lysate were diluted with 1.8 mL binding buffer A to D, to adjust each sample to the respective pH being tested. To avoid clogging of the membranes in the Vivapure® Mini spin columns, samples were clarified by passage through 0.45 µm CA Minisart NML syringe filters (Sartorius).

4 x Q and 4 x S Vivapure® Mini spin columns were labeled 4, 6, 8 and 10, corresponding to the pH of the buffer to be used. To each spin column, 400 µL of the corresponding binding buffer was added and spun for 5 minutes at 2,000 *g* (45° fixed angle rotor).

400 µL of the clarified samples adjusted to pH 4, 6, 8 or 10 were applied to each of the correspondingly equilibrated Vivapure® Q and S spin columns. Columns were spun for 5 min at 2,000 *g*.

Afterwards, Vivapure® Mini spin columns were reloaded with 400 µL sample and spun again for 5 min at 2,000 *g*. Loosely bound proteins were washed away with the application of 400 µL of the respective binding buffer to each of the columns and spinning for 5 min at 2,000 *g*. Flow-through and wash fractions were collected for subsequent detection of the target protein.

200 µL of elution buffer E, F, G or H, were applied to the washed columns and spun for 3 min at 2,000 *g*. Eluates were saved for subsequent analysis.

4 µL of flow-through, wash, and eluate fractions from each column were analyzed by reducing SDS-PAGE followed by silver staining.

Optimizing Elution Conditions

Taking account of the results of the first experiment (Scouting Binding Conditions) 200 µL cell lysate was diluted with 1.8 mL binding buffer B (25 mM KPi, pH 6). To avoid clogging of the membrane in the Vivapure® Mini spin column, the pH adjusted sample was clarified by passage through a 0.45 µm CA Minisart NML syringe filter (Sartorius).

400 µL binding buffer B was applied to one Vivapure® S Mini spin column and spun for 5 minutes at 2,000 *g*.

400 µL of the clarified sample was applied to the equilibrated Vivapure® S column and spun for 5 min at 2,000 *g*. Afterwards, the Vivapure® S Mini spin column was reloaded with 400 µL sample and spun again for 5 min at 2,000 *g*.

Loosely bound proteins were washed away by application of 400 µL binding buffer to the column and spinning for 5 min at 2,000 *g*. Flow-through and wash fractions were saved for analysis.

To elute the target protein, 100 µL elution buffer F, supplemented with 0.2 M NaCl was applied to the Vivapure® S Mini spin column and spun for 3 min at 2,000 *g*. The eluate was collected. For the next elution step, 100 µL of elution buffer F, supplemented with 0.4 M NaCl was applied and again spun for 3 min at 2,000 *g*. Elution was continued with 0.2 M NaCl increments until a final salt concentration of 1 M was reached, saving the eluates from each step.

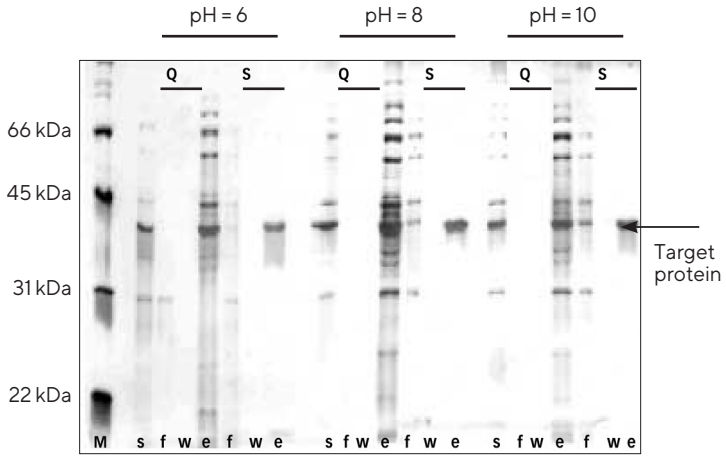
4 µL of flow-through, wash, and eluate fractions from each column were analyzed by reducing SDS-PAGE followed by silver staining.

Results

Dilution of the *E. coli* lysate with binding buffer A (25 mM Citrate, pH 4) lead to complete precipitation of sample proteins. Thus, pH 4 could not be tested in this experiment. As can be seen on the SDS gel in (Figure 1), the target protein was present in the eluates from Vivapure® Q Mini spin columns at all pH values tested, together with most of the *E. coli* proteins (Lanes Q "e"). In contrast, using the Vivapure® S Mini spin column, at all pH values tested, most *E. coli* proteins did not bind to the membrane and were found in the flow-through (Lane S "f"), thus resulting in purer target protein in all eluate fractions (Lane S "e").

Differences could be detected in the binding efficiency of the target protein. At pH 8, traces of the target protein were already found in the flow-through, with slightly higher amounts at pH 10 (Lane S "e"). At pH 6, the most efficient binding of the target protein to the S membrane was observed.

Figure 1

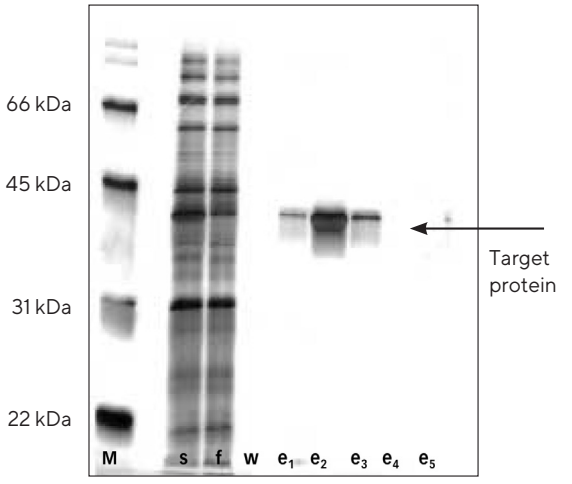


Sample	Sample Volume (µL)	Volume Loaded Onto Gel (µL)
M = Broad range marker		
s = Sample before application	800	4
f = Flow-through	800	4
w = Wash fraction	400	4
e = Elution with 1 M NaCl	200	4

Note: Scouting for optimal binding conditions of a SH2 domain expressed in *E. coli*. 12% reducing SDS gel, silver stained, shows the sample before purification (s), flow-through (f), wash (w) and eluate (e) fractions (1 M NaCl) from Vivapure® Q and S Mini spin columns, at the various pH values tested.

The purification conditions determined for Vivapure® S with potassium phosphate buffer (pH 6) were further optimized to determine the ideal salt concentration for SH2 domain elution. The target protein started to elute with 200 mM NaCl, however the main fraction eluted with 400 mM NaCl. Traces of the target protein were also found in the next elution step with 600 mM NaCl, but this might be due to the low elution volume.

Figure 2



Sample	Process Volume (µL)	Volume Loaded Onto Gel (µL)
M = Broad range marker		
s = Sample before application	800	16
f = Flow-through	800	16
w = Wash fraction	400	16
e1 = 25 mM KPi, pH 6, 200 mM NaCl	100	8
e2 = 25 mM KPi, pH 6, 400 mM NaCl	100	8
e3 = 25 mM KPi, pH 6, 600 mM NaCl	100	8
e4 = 25 mM KPi, pH 6, 800 mM NaCl	100	8
e5 = 25 mM KPi, pH 6, 1 M NaCl	100	8

Note: Optimizing elution conditions for a SH2 domain expressed in *E. coli,* using Vivapure® S Mini spin column at pH 6. 12% reducing SDS gel, silver stained, shows the sample before purification (s), flow through (f), wash (w) and eluate (e1-5) fractions.

Conclusion

A two-step procedure was used to rapidly scout optimal purification conditions for a target protein (a SH2 domain from *E. coli* lysate) with ion exchange chromatography. In the first step, the most suitable ion exchanger and buffer pH for binding the target protein was verified. In the second step, the elution condition was optimized, building on the results gained in step one. With the scouting procedure described here, it was possible to quickly and conveniently purify the target protein to homogeneity.

The results obtained in this experiment can be used to various ends, e.g:

- Polishing a specific protein after purification with another chromatographic technique
- Quickly establishing a FPLC method for a new protein
- Identification of the optimal purification method prior to scale up with Vivapure IEX Maxi spin columns.

For these purposes Vivapure IEX Mini and Maxi spin columns and Sartobind membrane adsorber units with FPLC connectors are available.

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