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Vivaflow® and Vivaspin® Workflow in Protein Research Laboratories

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Abstract

In this Application Note, we demonstrate how the Vivaflow® cassettes, Vivapure® Ion Exchange spin columns and Vivaspin® devices can be used to perform a complete protein purification workflow, from concentration and diafiltration of the original protein source, a cell culture supernatant, to final concentration | desalting of the purified protein. The protocol shows in detail the recoveries after each step along with the time needed for every purification and concentration step.

Introduction

Efficiency and efficacy of a multiple cycle experimental procedure was performed using Vivaflow® tangential flow cassettes for initial concentration and diafiltration of a cell culture supernatant. This was followed by Vivapure® Ion Exchange spin columns for the protein purification step and finally Vivaspin® 20 ultrafiltration devices for the final sample concentration and desalting. An artificial mixture of proteins in a RPMI-1640 culture medium was created to mimic the type of product that many researchers culture using e.g. the UniVessel device. This procedure further reflects a method that can be adapted to a large number of protein purification protocols, selecting alternative MWCOs and device sizes where necessary.

*Sample colour representative only

Methods

Part 1 – Creating and concentrating the culture medium
2 bottles (4 g) of RPMI-1640 were dissolved into 1.8 L dd-H_2O and 4 g of sodium acetate was added.

The pH was adjusted to 7.2 using 1M HCl. 2 g of BSA and 1 g of Lysozyme was added as protein samples, meant to be separated by chromatography. The volume of the cell culture supernatant sample was brought up to 2 L using dd-H_2O. After every preparation, concentration and purification step, 1 mL sample was set aside for SDS gel analysis.

Ion exchange chromatography was the method of choice for purifying lysozyme from the cell culture supernatant, especially from the "contaminant" BSA. For this, the 2 L cell culture supernatant needed to be concentrated and then diafiltered to adjust the sample to the starting conditions needed for the ion exchange chromatography binding step.

For concentration and diafiltration, the Vivaflow® 200 was used with a 5 kDa MWCO PES membrane. Vivaflow® 200 is a ready-to-use laboratory crossflow cassette in an acrylic housing, which allows caustic cleaning and 4-5 re-uses. Two cassettes can be run in parallel for the concentration of up to 5 L sample volumes. For the 2 L sample to be concentrated in this experiment, one cassette was sufficient. A Masterflex pump with an Easy-Load, size 16 pump head was used to run the Vivaflow® 200 cassette. Figure 1a. and 1b. show the Vivaflow® 200 set up with one or two cassettes.

The Vivaflow® 200 system was operated at 3 bar. Once 1.8 L of filtrate had been collected, the pump was stopped, the tubes removed from the cell culture medium concentrate and filtrate and the Vivaflow® system was purged with dd-H_2O. This solution now contained a 10-fold concentration of the constituent proteins from the original culture medium.

A BCA protein detection assay conveyed a 100% recovery of protein after this first concentration step. Table 1 indicates the time needed for the sample concentration.

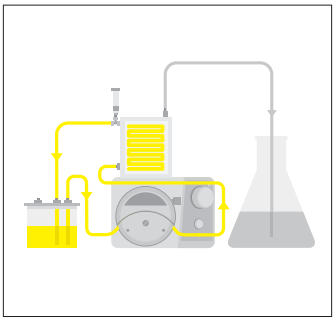

Fig. 1a. Vivaflow® 200 – single cassette

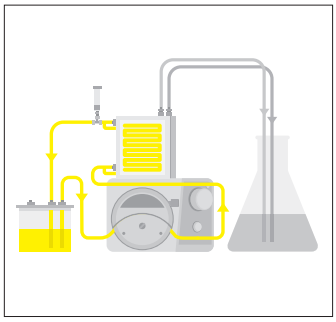

Fig. 1b. Vivaflow® 50R – two cassettes

Table 1: Vivaflow® 200, 5 kDa MWCO PES concentration speed.

Filtrate Volume (mL)	Time (hr:min:sec)
0	0:00:00
100	0:03:16
200	0:06:50
300	0:10:45
400	0:14:38
500	0:18:36
600	0:22:43
700	0:26:57
800	0:31:14
900	0:36:01
1,000	0:40:50
1,100	0:45:46
1,200	0:50:36
1,300	0:55:32
1,400	1:00:24
1,500	1:05:26
1,600	1:10:28
1,700	1:15:52
1,800	1:21:50

Part 2 – Buffer exchange of culture medium concentrate

The Vivaflow® 200 system was used for fast and easy diafiltration. To this end, the diafiltration reservoir, a Vivaflow® accessory, was filled with the 200 mL concentrated sample. Figure 2 shows the diafiltration set up. The Vivaflow® 200 system was set up as before, however attaching an additional tube to the diafiltration lid and placing this new inlet tube into a 25 mM sodium acetate (pH 5.5) buffer (needed to re-adjust the sample concentrate to the ionic starting conditions of the ion exchange chromatography step which was to follow). This enables concentration of the sample in the reservoir to the extent that the original buffer is removed and collected as waste (filtrate). Simultaneously, new buffer (25 mM sodium acetate) is drawn into the closed system, gradually leading to a buffer exchange while keeping the sample volume constant at 200 mL. The system was run at 3 bar. Once 1 L of buffer had been exchanged, diafiltration was stopped.

The 200 mL solution now contained the correct buffer to maintain the stability of the proteins of interest for the next part of the protocol and had the correct pH and salt concentration for the ion exchange binding step. BCA protein quantification again showed a 100% protein recovery.

Table 2 shows the time needed for diafiltration of 200 mL sample against 1,000 mL exchange buffer, again using Vivaflow® 200 with a 5 kDa MWCO PES membrane.

Fig. 2: Diafiltration system set up for buffer exchange. Culture medium concentrate can be seen in the center of the image. 1 L 25 mM sodium acetate (exchange buffer) can be seen connected to the system on the left of the image.

Filtrate Volume (mL)	Time (hr:min:sec)
0	0:00:00
100	0:06:58
200	0:14:16
300	0:22:39
400	0:29:40
500	0:37:02
600	0:44:15
700	0:51:34
800	0:58:54
900	1:06:03
1,000	1:13:02

Table 2: Diafiltration of 200 mL concentrated cell culture supernatant containing the proteins lysozyme and BSA against 1,000 mL 25 mM sodium acetate.

Part 3 – Purification of Lysozyme, the protein of interest

The purification of Lysozyme was performed using a Vivapure® cation exchange membrane adsorber device (Vivapure® Maxi H S). The membrane adsorber matrix holds the active ligands and performs like a traditional cation exchanger. However, membrane adsorbers represent a special form of chromatography matrix. Unlike traditional resins, they make use of convective transport to bring proteins to the ion exchange surface; hence, binding, washing and elution is performed quickly and high binding capacities are achieved, even at high flow rates. This allows their use in fast and convenient centrifugal spin columns (Fig. 3).

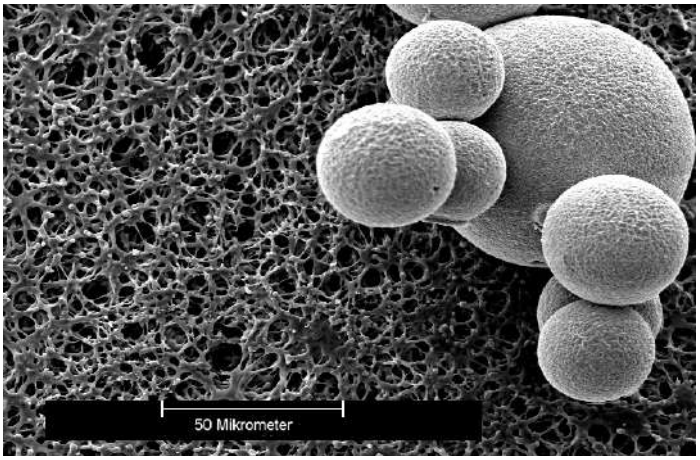

Fig. 3: Electron micrograph of chromatography gel beads (upper right) in comparison to a Q ion exchange membrane adsorber (background), revealing 100-fold larger pore sizes of the membrane adsorber.

Two Vivapure® Maxi H S type devices (Fig. 4) were each equilibrated with 10 mL of 25 mM sodium acetate (pH 5.5), by filling with 10 mL of this buffer and centrifuging for 5 min. in a swing bucket centrifuge at 500 *g* and discarding the flow through. Using the concentrated and buffer exchanged sample from Part 2, 10 mL samples were pipetted into each of the equilibrated Vivapure® devices and centrifuged again for 5 min. in a swing bucket centrifuge at 500 *g*. The Vivapure® devices were washed with 10 mL of 25 mM sodium acetate, discarding the flow through, followed by an elution step with 5 mL of 1 M NaCl in 25 mM sodium acetate. A BCA assay revealed 95% lysozyme recovery.

Fig. 4: Vivapure® Maxi spin columns can be used in a centrifuge for fast and easy protein purification.

The eluate was then concentrated in a Vivaspin® 20 (5 kDa MWCO PES), Figure 5, and centrifuged at 5,000 *g* for 10 min. or until approximately 2 mL of concentrate had been collected. The device was then re-filled with 18 mL 50 mM potassium phosphate buffer (pH 7.2) to 20 mL for a final buffer exchange and desalting of the purified sample. The sample was again centrifuged until a final sample volume of 2 mL had been attained. A BCA assay revealed 97% lysozyme recovery.

Fig. 5: Vivaspin® 20 ultrafiltration device, on the right with a pressure cap which allows pressurization of the device as an alternative to the regular centrifugal operation.

Part 4 – Analyzing the samples

The samples of the individual steps were analyzed by SDS gel, using reducing sample buffer (prepared by adding 50 µL 2-mercaptoethanol to 950 µL Laemmli sample buffer). For all steps, 5 µL of the 1 mL sample taken during the experiment were diluted with 95 µL reducing sample buffer, of which 20 µL were loaded onto a 12% Tris-HCl SDS gel (Fig. 6).

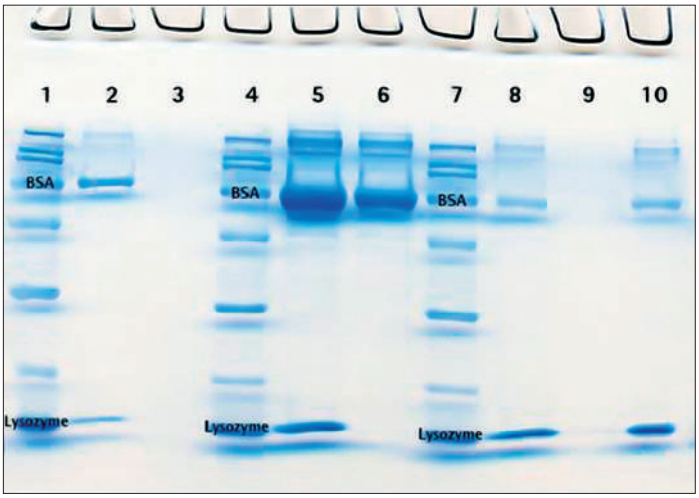

Fig. 6: Coomassie stained 12% Tris-HCl SDS gel loaded with 20 µL sample preparations. Lane 1: Marker (SDS Broad Range); Lane 2: Original sample; Lane 3: Original sample filtrate (Part 1); Lane 4: Marker; Lane 5: Buffer exchange concentrate (Part 2); Lane 6: Filtrate after binding (Part 3); Lane 7: Marker; Lane 8: Filtrate after elution (Part 3); Lane 9: Filtrate after concentrating and desalting (Part 3); Lane 10: Concentrate after concentrating and desalting.

Conclusion

The overall result shows that a standard and straightforward procedure can be followed to concentrate, purify, isolate and analyze a protein of interest from a cell culture, using Vivaflow® 200 tangential flow units for cell culture supernatant concentration and diafiltration, Vivapure® for ion exchange chromatography, followed by Vivaspin® 20 for final sample concentration and desalting.

In many cases dialysis, which is an overnight procedure would be performed instead of the much quicker alternative - ultrafiltration. Here, we show how time-saving and efficient ultrafiltration is for diafiltration and desalting applications, as well as for protein concentration.

The set up and completion of protein purification takes approx. 3.45 h using this method, starting from a culture supernatant, with high protein recoveries in each step (see Table 3). A total protein purification procedure can therefore be completed within 1 working day, including SDS gel analysis, utilizing this strategy, when adapted to individual needs.

Table 3: Processing times for a complete protein purification workflow.

Task	Time	Recovery
Vivaflow® 200 set up and concentration	1 hour 25 min.	100%
Vivaflow® 200 set up and diafiltration	1 hour 20 min.	100%
Vivapure® purification	45 min.	95%
Vivaspin® concentration and desalting	30 min.	97%
Total	3 hours 45 min.	92%

Products used in this experiment	Order No.
Vivaflow® 200, 5 kDa MWCO PES	VF20P1
500 mL Diafiltration Reservoir	VFA006
Vivapure® S H Maxi	VS-IX20SH08
Vivaspin® 20, 5 kDa MWCO PES	VS2011

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