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Application Note

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Desalting and Buffer Exchange with Vivaspin® Centrifugal Concentrators

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Abstract

This short application note highlights the ability to reduce protein sample salt concentrations by up to 99%, or to exchange the buffer sample entirely, using Vivaspin® 20 and Vivaspin® 6 centrifugal ultrafiltration devices. This process is known as diafiltration and prevents the over-concentration of proteins with a tendancy to precipitate at higher salt concentration. Furthermore, in comparison to conventional re-buffering techniques such as dialysis, a complete diafiltration process can typically be performed in a matter of minutes, instead of 1 - 2 days or more.

Introduction

Vivaspin® centrifugal concentrators, with patented vertical membrane technology, combine fast filtration with high recovery of target proteins. This makes Vivaspin® the technology of choice for desalting or buffer exchange, avoiding lengthy dialysis steps.

While proteins are retained by an ultrafiltration membrane, salts can pass freely through, independent of protein concentration or membrane MWCO. In consequence, the composition of the buffer in the flow-through and retentate is unchanged after protein concentration. By diluting the concentrate back to the original volume, the salt concentration is lowered. The concentrate can be diluted with water or salt-free buffer if simple desalting is required; however, it is also possible to dilute the concentrate with a new buffer, thereby exchanging the buffering substance entirely. For example, a 10 mL protein sample containing 500 mM salt, if concentrated 100-fold still contains 500 mM salt. If this concentrate is then diluted 100× with water or salt-free buffer, the protein concentration returns to the original level, while the salt concentration is reduced 100× to only 5 mM (i.e. a 99% reduction in salt concentration).

The protein sample can then be concentrated again to the desired level, or the buffer exchange can be repeated to reduce the salt concentration even further before a final concentration of the protein. This process is called diafiltration. For proteins with a tendency to precipitate at higher concentrations, it is possible to perform several diafiltration steps in sequence, with the protein concentrated each time to only 5 or 10x. For example, if a precipitous protein sample is concentrated to 5x then diluted back to the original volume, and this process is repeated a further two times, this still results in a >99% reduction in salt concentration, without over-concentrating the protein.

Methods

Select an appropriate MWCO for your sample.
For maximum recovery, select a MWCO ⅓ to ½ the molecular weight of the molecule of interest.

1. Add protein sample up to the maximum fill volume of the concentrator (as stated in the device operating instructions). If the sample volume is lower than the maximum device volume, it can be diluted to the maximum fill volume before the first centrifugation step. This will increase the salt removal rate.
2. Centrifuge for the recommended amount of time at an appropriate spin speed (see device operating instructions).
3. Empty filtrate container and refill the concentrator with an appropriate exchange solvent.*
4. Centrifuge again as before.
5. Recover the concentrated, desalted sample from the bottom of the concentrate pocket with a pipette.

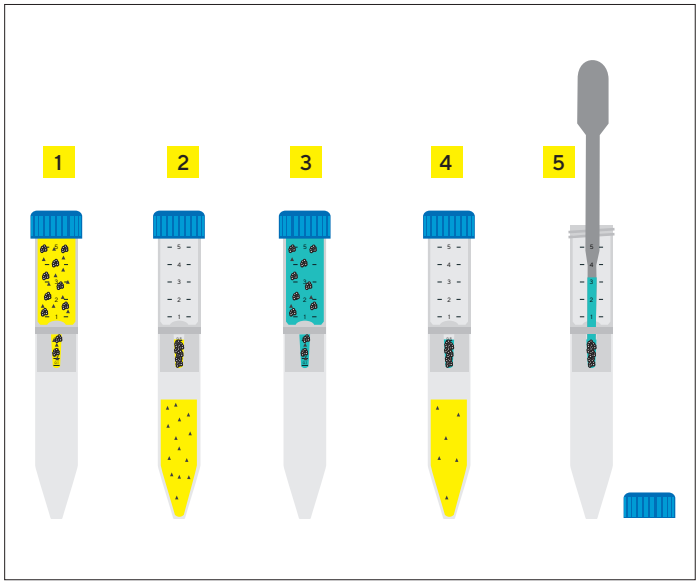

Figure 1: Step-by-step method for desalting and concentration

* Filtrate volumes should be retained until the concentrated sample has been analyzed.

Results

Vivaspin® 20

MWCO	5 kDa		30 kDa		50 kDa		100 kDa	
	Cytochrome C 0.25 mg/mL		BSA 1 mg/mL		BSA 1 mg/mL		IgG 1 mg/mL	
	Protein Recovery	NaCl Removal	Protein Recovery	NaCl Removal	Protein Recovery	NaCl Removal	Protein Recovery	NaCl Removal
Spin 1	100%	99%	97%	99%	97%	99%	90%	98%
Spin 2	96%	100%	92%	100%	93%	100%	87%	100%

Four Vivaspin® 20 devices of each MWCO were tested with 20 mL samples. Each sample contained 500 mM NaCl. To perform diafiltration, devices were centrifuged at 4,000 *g* for 45 min (5 kDa MWCO) or 30 min (>5 kDa MWCOs).

After the first and second spins, the retentate samples were brought up to 20 mL with ultrapure water from an Arium® system (Sartorius). OD readings were taken at 410 nm for Cytochrome C or 280 nm for BSA and IgG samples. Salt concentrations were measured using a Qcond 2200 conductivity measuring instrument.

Vivaspin® 6

MWCO	5 kDa		30 kDa		50 kDa		100 kDa	
	Cytochrome C 0.25 mg/mL		BSA 1 mg/mL		BSA 1 mg/mL		IgG 1 mg/mL	
	Protein Recovery	NaCl Removal	Protein Recovery	NaCl Removal	Protein Recovery	NaCl Removal	Protein Recovery	NaCl Removal
Spin 1	98%	99%	92%	99%	93%	99%	92%	98%
Spin 2	85%	100%	86%	100%	83%	100%	89%	100%

Four Vivaspin® 6 devices of each MWCO were tested with 6 mL samples. Each sample contained 500 mM NaCl. To perform diafiltration, devices were centrifuged at 4,000 *g* for 45 min (5 kDa MWCO) or 30 min (>5 kDa MWCOs).

After the first and second spins, the retentate samples were brought up to 6 mL with ultrapure water from an Arium® system (Sartorius). OD readings were taken at 410 nm for Cytochrome C or 280 nm for BSA and IgG samples. Salt concentrations were measured using a Qcond 2200 conductivity measuring instrument.

Conclusions

As the results show, the efficient design of Vivaspin® devices allowed >95% of the salt to be removed during the first centrifugation step. Only one subsequent centrifugation step was needed to increase the typical salt removal to 99% with >92% recovery of the target protein.

Diafiltration using ultrafiltration devices such as Vivaspin® 6 and 20 represents a faster and more efficient solution to desalting and buffer exchange, than conventional techniques such as dialysis.

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