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Desalting and Buffer Exchange with Vivaspin® Centrifugal Concentrators

Application Note

#04
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turning science into solutions

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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 appropriate 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 100x still contains 500 mM salt. If this concentrate is then diluted 100x with water or salt-free buffer, the protein concentration returns to normal, while the salt concentration is reduced 100x to only 5 mM, (I.E. a 99% reduction in salt).

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.

Desalting and Buffer Exchange Procedure
(See Figure 1.)

1. Select the most appropriate MWCO for your sample. For maximum recovery, select a MWCO ½ to ⅓ the molecular size of the species of interest.

2. Fill concentrator with up to the maximum volume stated in the device operating instructions*, (e.g. 20 ml if Vivaspin® 20 is used).

3. If the sample is smaller than the maximum device volume*, it can be diluted up to the maximum volume before the first centrifugation step. This will help increase the salt removal rate.

4. Centrifuge for the recommended amount of time at an appropriate spin speed for your Vivaspin® model*.

5. Empty filtrate container†.

6. Refill concentrator with an appropriate solvent.

7. Centrifuge again as before.

8. Empty filtrate container†.

9. Recover the concentrated, de-salted sample from the bottom of the concentrate pocket with a pipette.

Notes
* For guidance on maximum fill volumes, spin speeds and suggested spin times, please refer to the Operating Instructions that accompany your Vivaspin® products.

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

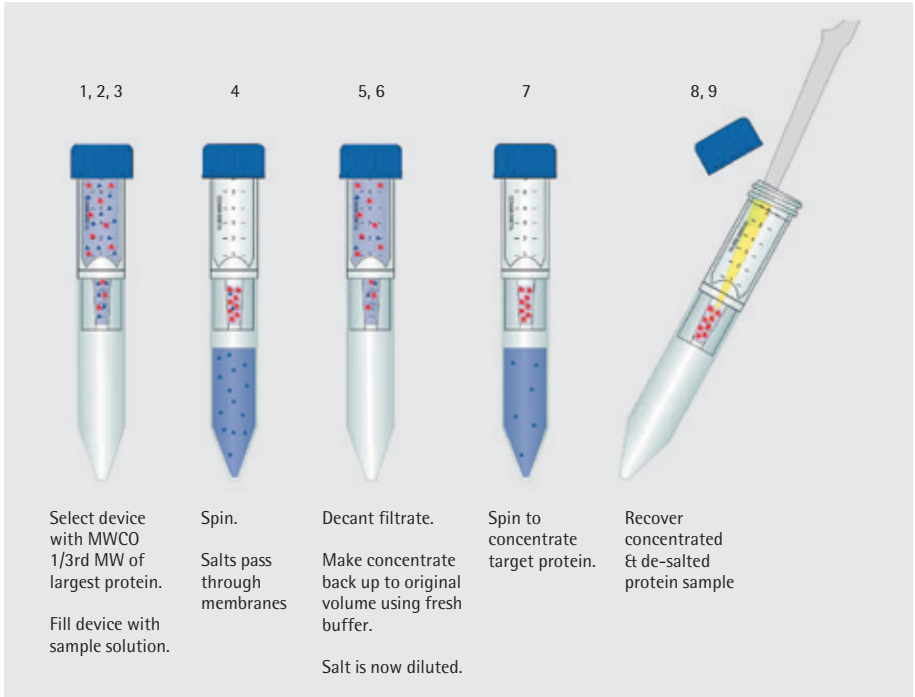

Figure 1: Step-by-step method for desalting and concentration

Test Results
As the results below 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 sample.

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 cut-off were tested with 20 ml of solution. Each of the solutions contained 500 mM NaCl. Each spin was performed at 4,000 × g. The devices > 5kDa were spun for 30 min. The devices with 5 kDa were spun 45 min. After the first and second spin, the retentate was brought up to 20 ml with ultra pure water from the Arium system (Sartorius). OD readings were taken at 410 nm for the Cytochrome C and 280 nm for the BSA and IgG samples. Salt concentration was measured with 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 cut- off were tested with 6 ml of solution. Each of the solutions contained 500 mM NaCl. Each spin was performed at 4,000 × g. The devices > 5 kDa were spun for 30 min. The devices with 5 kDa were spun 45 min. After the first and the second spin the retentate was brought up to 6 ml with ultra pure water from the Arium system (Sartorius) OD readings were taken at 410 nm for the Cytochrome C and 280 nm for the BSA and IgG samples. Salt concentration was measured with a Qcond 2200 conductivity measuring instrument.

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