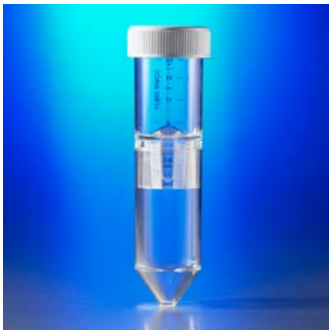
Desalting and Buffer Exchange with Corning's Spin-X® UF Concentrators

Protocol

CORNING

Spin-X UF 500

Spin-X UF 6



Spin-X UF 20

Introduction

Spin-X UF concentrators, with vertical membrane technology combine fast filtration with high recovery of target proteins. This makes Spin-X UF 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 molecular weight cut-off (MWCO). Consequently, 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 concentrated 100-fold still contains 500 mM salt. If this concentrate is then diluted 100-fold with water or salt-free buffer, the protein concentration returns to normal, while the salt concentration is reduced 100-fold 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 10-fold. For example, if a precipitous protein sample is concentrated 5-fold then diluted back to the original volume and this process is repeated a further two times, this still results in a greater than 99% reduction in salt concentration without over-concentrating the protein.

Desalting and Buffer Exchange Procedure

1. Select the most appropriate MWCO for your sample. For maximum recovery, select a MWCO half to one-third the molecular size of the species of interest.
2. Add the solution to be desalted to the upper chamber of the Spin-X UF concentrator. See Table 1 to determine maximum volume, and check the instructions that came with the concentrator.
3. If the sample is smaller than the maximum concentrator volume, it can be diluted up to the maximum volume with the replacement buffer 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 Spin-X UF concentrator (Table 1). When fixed angle rotors are used, angle the concentrator so that the printed window faces upwards (outwards). **NOTE**: This step concentrates the proteins in the solution remaining in the upper chamber, but does not change the salt concentration in either chamber.

Table 1. Maximum Recommended Centrifugal Force for Spin-X® UF Concentrators

Concentrator	Spin-X UF 500	Spin-X UF 6	Spin-X UF 20
Maximum Spin Force – Swing Bucket			
5,000 to 50,000 MWCO PES	Do not use	4,000 xg	4,000 xg
>100,000 MWCO PES	Do not use	4,000 xg	3,000 xg
Maximum Spin Force – Fixed Angle			
5,000 to 50,000 MWCO PES	12,000 xg	8,000 xg	6,000 xg
>100,000 MWCO PES	12,000 xg	6,000 xg	6,000 xg

5. Empty the lower filtrate container which should now contain the salt or buffer solution minus the proteins. **NOTE**: Retain filtrate until the concentrated sample has been analyzed.
6. Refill the upper concentrator chamber with an appropriate replacement solvent or buffer. This dilutes both the proteins and remaining salts.
7. Centrifuge again (Step 4). **NOTE**: This step concentrates the proteins in the solution remaining in the upper chamber while removing almost all the salt.
8. Empty filtrate container. **NOTE**: Retain filtrate until the concentrated sample has been analyzed.
9. Recover the concentrated, desalted sample from the bottom of the concentrate pocket in the upper chamber with a pipettor fitted with a fine tip.

Optimizing Solute Recovery

When highest solute recoveries are most important, in particular when working with solute quantities in the microgram range, Corning recommends considering the following key points:

- Select the smallest concentrator that suits the sample volume. Additionally, take advantage of the extra speed of Spin-X UF concentrators by refilling a smaller concentrator repeatedly.
- Select the lowest MWCO membrane that suits the application.
- When available, use swing bucket rotors rather than fixed angle rotors (except for the Spin-X UF 500 which must always be run in a fixed angle rotor). This reduces the surface area of the concentrator that will be exposed to the solution during centrifugation.
- Reduce centrifugal force to approximately half of the maximum recommended (Table 1).
- Avoid over-concentration. The smaller the final concentrate, the more difficult it is to achieve complete recovery. If feasible, after a first recovery, rinse the device with one or more drops of buffer and then recover again.

Test Results

As the results show (Tables 2 and 3), the efficient design of Spin-X UF concentrators allowed greater than 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 greater than 92% recovery of the sample.

Spin-X UF 20 Concentrator Results

Table 2. Results of Desalting Procedure Using Spin-X UF 20 Concentrators

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 Spin-X® UF 20 concentrators 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 xg. The devices with greater than 5kDa MWCO were spun for 30 minutes. Concentrators with 5 kDa MWCO were spun 45 minutes. After the first and second spin, the retentate was brought up to 20 mL with ultrapure water from an Arium® purification 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 instrument.

Spin-X UF 6 Concentrator Results

Table 3. Results of Desalting Procedure Using Spin-X UF 6 Concentrators

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 Spin-X UF 6 concentrators 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 xg. The concentrators with MWCO greater than 5 kDa were spun for 30 minutes. Concentrators with 5 kDa cut-offs were spun 45 minutes. After the first and the second spin, the retentate was brought up to 6 mL with ultrapure water from an Arium purification 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 instrument.

Ordering Information

Spin-X UF Concentrators

Cat. No.	Description	Capacity	Membrane (MWCO)	Qty/Cs
431477	Spin-X UF 500	500 µL	5,000	25
431478	Spin-X UF 500	500 µL	10,000	25
431479	Spin-X UF 500	500 µL	30,000	25
431480	Spin-X UF 500	500 µL	50,000	25
431481	Spin-X UF 500	500 µL	100,000	25
431482	Spin-X UF 6	6 mL	5,000	25
431483	Spin-X UF 6	6 mL	10,000	25
431484	Spin-X UF 6	6 mL	30,000	25
431485	Spin-X UF 6	6 mL	50,000	25
431486	Spin-X UF 6	6 mL	100,000	25
431487	Spin-X UF 20	20 mL	5,000	12
431488	Spin-X UF 20	20 mL	10,000	12
431489	Spin-X UF 20	20 mL	30,000	12
431490	Spin-X UF 20	20 mL	50,000	12
431491	Spin-X UF 20	20 mL	100,000	12

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