

Treatment of Spin-X[®] UF Concentrators for Improved Recovery of Low-Concentrated Protein Samples

Protocol

CORNING



Spin-X UF 500 concentrator



Spin-X UF 6 concentrator



Spin-X UF 20 concentrator

Introduction

With appropriate device size and membrane cut-off selected, Spin-X UF concentrators will typically yield recoveries for the concentrated sample greater than 90% when the starting sample contains over 0.1 mg/mL protein of interest.

Although dependent on the sample characteristics, solute (protein) adsorption on the polyethersulfone (PES) membrane surface is typically very low (2 to 10 $\mu\text{g}/\text{cm}^2$) and in practice is not detectable. Typically, a higher molecular cut-off (MWCO) membrane will bind more solute than a low MWCO membrane. Sample losses through adsorption can increase to 20 to 100 $\mu\text{g}/\text{cm}^2$ when a solute in the filtrate is of interest and it must pass through the whole internal structure of the ultrafiltration (UF) concentrator membrane. While the relative adsorption to the plastic surface of the sample container will be proportionately less than on the membrane (due to the membrane's higher total internal surface area), binding to the plastic surface of the upper and lower chambers can still be a source of yield loss. Whenever possible, choose the lowest MWCO membrane in the smallest concentrator applicable. Swinging bucket rotors are preferred to fixed angle rotors because they reduce the surface area of the concentrator that will be exposed to the solution during centrifugation.

An important factor not to be neglected is the thorough recovery of the retentate. Make sure to carefully remove all traces of solution from the upper sample container and, if feasible, rinse the device after recovering the sample with one or more drops of buffer, and then recover again.

The intention of the following passivation procedure is to improve recovery of protein samples in the nanogram to microgram concentration range by pretreating (passivating) the concentrator to block or coat its membrane and plastic surfaces to reduce their "stickiness" for the solute molecules of interest. For this purpose a range of solutions are suggested in Table 1.

Passivation Procedure for Spin-X UF Concentrators

1. Wash the concentrators once by filling with high purity water, and spin the liquid through
2. Remove residual water thoroughly by pipetting. **CAUTION: Take care not to damage the membrane with the pipet tip.**
3. Fill concentrators with the blocking solution of choice as given in Table 1.

Table 1. Passivation Solutions

Type	Concentration
Powdered milk	1% in high purity water
BSA	1% in PBS
Tween [®] 20	5% in high purity water
SDS	5% in high purity water
Triton [™] X-100	0.5% in high purity water
PEG 3000	5% in high purity water

4. Incubate the filled concentrators at room temperature for at least 2 hours (overnight is also possible except for Triton X-100 which is not recommended for overnight incubation).

5. Pour out the blocking solution.
6. Rinse the device 3 to 4 times very thoroughly with high purity water, and finally spin through.
7. The concentrators are now passivated or blocked and ready for use. We recommend evaluating different passivation reagents by comparing passivated concentrators side-by-side against untreated concentrators.

NOTE: It is necessary to rinse the concentrator thoroughly before each wash/spin to ensure that traces of passivation compound are entirely removed from the bottom of the upper chamber. Use the concentrator immediately for protein concentration or store it at 4°C filled with high purity water, to prevent the membrane from drying.

Ordering Information

Corning® Spin-X® UF Concentrator



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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