

Diafiltration Spin Column for Buffer Exchange & Desalting Protocol



Materials Required

Reagents	Catalog #	Equipment
Diafiltration spin columns	S-4004	Variable-speed bench-top microcentrifuge
Modification Buffer*	S-4003	UV-Spectrophotometer
Conjugation Buffer*	S-4002	

*Depending on desired final buffer

Buffer Exchange & Desalting Procedure

A. Protein Sample Loading

1. Vivaspin 500 diafiltration spin filters are made to contain and process volumes of 500 μL or less. If volumes greater than 500 μL are to be processed, then multiple filters or loadings may be required.
2. Add 50-500 μL to the diafiltration concentrator body (Figure 1) and place into the centrifuge with an appropriate balance. Make sure the concentrator body lid is closed.

If only protein concentration is needed, refer to section B. If buffer exchange / purification is required, skip to section C.

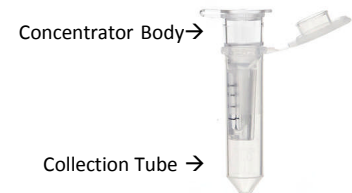


Figure 1: Diafiltration Spin Column

B. Protein Concentration

1. Centrifuge for 5 minutes at 5,000 x g.
2. After initial spin, check volume left in concentrator body.
 - a) If it is more than desired volume, pipette up and down to mix without touching the membrane and re-spin and repeat until desired volume is achieved.
 - b) When desired volume is achieved, remove solution from concentrator body and the protocol is completed.

C. Protein Purification & Buffer Exchange

1. Centrifuge for 5 minutes at 5,000 x g.
2. After the spin, check to make sure the volume is at 50 μL or less in the concentrator body.
 - a) If the volume is greater than 50 μL , pipette solution in concentrator body up & down to mix without touching the membrane. Then re-spin column and repeat until it reaches 50 μL .
 - b) A complete spin is achieved when the concentrator body volume reaches 50 μL or less.

NOTE: Some proteins cannot be concentrated to 50 μL before precipitation occurs from the potentially high concentration. If the protein has special concentration requirements, the volumes in this protocol should be adjusted accordingly.

3. The excess buffer will flow into the collection tube. Collect the flow through after each complete spin in a separate microcentrifuge tube. This is a precautionary step to ensure no protein is lost if the membrane breaks.
4. Add 450 μL of desired buffer to the concentrator body and pipette up and down to mix the solution without touching the membrane. Make sure not to touch or damage the filter surface with the pipette tip.
5. Repeat steps 5 to 8 an additional four complete spin times.
6. After the last spin, transfer the protein to a new microcentrifuge tube.