INSTRUCTIONS



Zeba Spin Desalting Columns and Plates, 7K MWCO

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Number	Description				
89877	Zeba Spin Desalting Columns, Micro (75μL), 25 columns, for 2-12μL samples				
89878	Zeba Spin Desalting Columns, Micro (75μL), 50 columns, for 2-12μL samples				
89882	Zeba Spin Desalting Columns, 0.5mL, 25 columns, for 30-130μL samples				
89883	Zeba Spin Desalting Columns, 0.5mL, 50 columns, for 30-130μL samples				
89889	Zeba Spin Desalting Columns, 2mL, 5 columns, for 200-700µL samples				
89890	Zeba Spin Desalting Columns, 2mL, 25 columns, for 200-700μL samples				
89891	Zeba Spin Desalting Columns, 5mL, 5 columns, for 500-2000µL samples				
89892	Zeba Spin Desalting Columns, 5mL, 25 columns, for 500-2000µL samples				
89893	Zeba Spin Desalting Columns, 10mL, 5 columns, for 700-4000µL samples				
89894	Zeba Spin Desalting Columns, 10mL, 25 columns, for 700-4000µL samples				
89807	Zeba Spin Desalting Plates, 96-well, 2 plates				
89808	Zeba Spin Desalting Plates, 96-well, 4 plates				
	Note: These products are recommended for processing compounds > 7000 Da. The resin slurry is supplied in 0.05% sodium azide.				
	Storage: Upon receipt store at 4°C. Product shipped at ambient temperature.				

Introduction

The Thermo ScientificTM ZebaTM Spin Desalting Columns contain a high-performance resin that offers exceptional desalting or buffer-exchange for protein samples. Samples volumes between $2\mu L$ -4mL and containing as low as $20\mu g$ of protein/mL can be processed with unsurpassed protein recovery and $\geq 95\%$ retention of salts and other small molecules (< 1000Da). These columns require no chromatography system. The spin-column method eliminates waiting for samples to emerge by gravity flow and the subsequent monitoring of fractions for protein recovery.

Important Product Information

- The Zeba Spin Desalting Columns and Plates contain a size-exclusion chromatographic resin to separate proteins from small molecules. As with all size exclusion-based separation, the amount of small molecule removed and protein recovered are affected by the nature of the molecules and volume of sample. The sample volumes recommended provide exceptional removal of a variety of small molecules (typically > 95% for molecules < 1000Da); however, proteins and small molecules often behave differently than predicted because of a variety of factors such as hydrophobicity, secondary structure and interactions. Therefore, some optimization of sample volume might be required to achieve optimal performance for each specific sample. In general, reducing the sample volume added to the column increases small molecule removal, and increasing sample volume maximizes protein recovery.
- Also available are Zeba Spin Desalting Columns and Plates with a 40K MWCO, which enable removal of salts and other small molecules < 2000Da and recovery of proteins and other macromolecules > 40,000Da.



Procedure for Desalting or Buffer Exchange

Additional Materials Required

- For 75μL and 0.5mL spin columns: Bench-top microcentrifuge (1500 × g) and 1.5mL microcentrifuge tubes
 Note: Use a centrifuge that can be adjusted to 1500 × g, such as the Thermo ScientificTM SorvallTM Legend Micro 17 Microcentrifuge
- For 2 and 5mL spin columns: Centrifuge $(1000 \times g)$ and 15mL conical tubes
- For 10mL spin columns: Centrifuge $(1000 \times g)$ and 50mL conical tubes
- For the desalting plates: Variable-speed centrifuge with rotor and carrier capable of handling stacked plates (height = 4.4cm) at 1000 × g
- Wash/equilibration buffer

Note: Use the same wash/equilibration (stacker) buffer as is desired for the final sample solution. Equilibrating the desalting resin before sample loading is necessary to ensure proper buffer exchange.

Procedure for Protein Desalting

Note: See **Table 1** for centrifugation times and volumes for the buffer, stacker and sample for each device. For maximum protein recovery, add a stacker on top of the applied sample for volumes below the volume specified in **Table 1**.

- 1. Remove the column's bottom closure or the plate's bottom sealing material. Loosen cap (do not remove cap).
- 2. Place the column into a collection tube or plate on top of a wash plate and centrifuge to remove the storage solution.
- 3. Discard flow-through and replace the column back into the collection device.
- 4. Add wash/equilibration buffer on top of the resin. Centrifuge device and discard flow-through. Repeat this step two additional times.

Note: After each spin, the resin should appear white and free of liquid. If liquid is present, make sure you are using the correct centrifugation speed and time. Incomplete centrifugation may result in poor sample recovery or sample dilution.

- 5. Blot the bottom of the column or plate to remove excess liquid. Transfer device to a new collection tube or plate.
- 6. Apply sample on top of the resin. If needed, add a stacker as soon as the sample has entered the resin. Adding a stacker is optional but recommended for dilute protein solutions or small sample volumes to ensure maximum sample recovery.
- 7. Centrifuge and retain flow-through that contains sample. Discard spin column or plate.

Table 1. Centrifugation times and volumes for the buffer, stacker and sample.

Column or Plate		75μL	<u>0.5mL</u>	2mL	5mL	<u>10mL</u>	Plate
Sample Volume Range (µL)		2-12	30-130	200-700	500-2000	700-4000	20-100
Wash/equilibration Buffer Volume		50μL	300µL	1mL	2.5mL	5mL	250μL
Sample Volume (µL)*		< 5	< 70	< 350	< 750	< 1500	< 30
Optional Stacker Volume (µL)*		3	15	40	100	200	10
Centrifuge Speed $(\times g)$		1000	1500	1000	1000	1000	1000
Centrifugation Time (min)	Storage Solution Removal	1	1	2	2	2	2
	Wash 1	1	1	2	2	2	2
	Wash 2	1	1	2	2	2	2
	Wash 3	1	1	2	2	2	2
	Sample Recovery	2	2	2	2	2	2

^{*}When using the indicated sample volumes, use a stacker to achieve the highest recovery. The stacker is a volume of wash/equilibration buffer applied after the added sample has completely entered the desalting resin bed.



Troubleshooting

Problem	Possible Cause	Solution			
Sample or buffer does not	Centrifugation problem	Ensure that centrifuge is in proper working condition			
flow through resin		Ensure bottom closure is removed			
		Ensure top cap is loosened			
Sample contamination	Improper sample loading	Apply sample directly to center of the resin bed; touch tip to resin to expel all sample			
		Avoid contact with sides of column			
	Improper centrifugation	For fixed-angle rotors, place column in the same orientation each time and do not exceed recommended centrifuge speed			
		Do not exceed recommended centrifugation speed or time			
Low yield	Sample was not completely in solution before adding to the column	Centrifuge sample at $14,000 \times g$ for 10 minutes before adding to the column			
	Portion of protein still remaining in spin column	Use a stacker to recover more protein (for most samples, the majority of protein is recovered without a stacker)			
	Protein precipitated in equilibration buffer	Check for protein solubility in the final buffer or solution			
	Protein bound to resin matrix	Use alternative resin, such as Polyacrylamide Spin Desalting Columns (see Related Thermo Scientific Products section)			
Recovered protein or sample is dilute	Wash/equilibration buffer was not adequately removed	Before adding the sample make sure the wash/equilibration buffer was adequately removed by centrifugation (i.e., column appears uniformly white with no solvent streaks)			

Related Thermo Scientific Products

89849 Pierce Polyacrylamide Spin Desalting Columns, 7K MWCO, 0.7mL, 25 columns
 89862 Pierce Polyacrylamide Spin Desalting Columns, 7K MWCO, 0.7mL, 50 columns

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(815) 968-0747

(815) 968-7316 fax