INSTRUCTIONS

Zeba™ Spin Desalting Columns and Plates, 40K MWCO

2190.3

Number	Description
87764	**Zeba Spin Desalting Columns, Micro (75µL),** 25 columns, for 5-14µL samples
87765	**Zeba Spin Desalting Columns, Micro (75µL),** 50 columns, for 5-14µL samples
87766	**Zeba Spin Desalting Columns, 0.5mL,** 25 columns, for 70-200µL samples
87767	**Zeba Spin Desalting Columns, 0.5mL,** 50 columns, for 70-200µL samples
87768	**Zeba Spin Desalting Columns, 2mL,** 5 columns, for 200-900µL samples
87769	**Zeba Spin Desalting Columns, 2mL,** 25 columns, for 200-900µL samples
87770	**Zeba Spin Desalting Columns, 5mL,** 5 columns, for 300-2000µL samples
87771	**Zeba Spin Desalting Columns, 5mL,** 25 columns, for 300-2000µL samples
87772	**Zeba Spin Desalting Columns, 10mL,** 5 columns, for 1000-4000µL samples
87773	**Zeba Spin Desalting Columns, 10mL,** 25 columns, for 1000-4000µL samples
87774	**Zeba Spin Desalting Plates, 96-well,** 2 plates
87775	**Zeba Spin Desalting Plates, 96-well,** 4 plates

Note: These products are recommended for processing compounds > 40,000 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 Scientific™ Zeba™ Spin Desalting Columns and Plates, 40K molecular-weight cutoff (MWCO) contain a high-performance resin that offers exceptional desalting and protein recovery characteristics. The Zeba 40K MWCO resin is for buffer exchanging proteins with molecular weight > 40K and removing small molecules < 2000Da. A variety of spin-column volumes are available for processing samples ranging from 5-4000µL (Table 1). The spin-column and plate formats provide exceptional ease of use while eliminating sample dilution common to gravity-flow methods.

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 1500-2000Da); 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 7K MWCO, which enable removal of salts and other small molecules < 1000Da and recovery of proteins and other macromolecules > 7000Da.

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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 Scientific™ Sorvall™ Legend Micro 17 Microcentrifuge

- For 2 and 5mL spin columns: Centrifuge (1000 × *g*) and 15mL conical tubes
- For 10mL spin columns: Centrifuge (1000 × *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: For maximum protein recovery, add a stacker on top of the applied sample. See Table 1 for centrifugation times and volumes for the buffer, stacker and sample.

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 tube.
4. Add wash/equilibration buffer on top of the resin. Centrifuge tube 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 column to a new collection tube or place plate on top of a collection 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 and recommended for dilute protein solutions to ensure maximum sample recovery.
7. Centrifuge and retain flow-through that contains sample. Discard spin column.

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

Column or Plate		75µL	0.5mL	2mL	5mL	10mL	Plate
Sample Volume Range (µL)		5-14	70-200	200-900	300-2000	1000-4000	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 (× *g*)		1500	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	2	3	4	6	3
	Sample Recovery	2	2	3	4	4	3

*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 flow through resin	Centrifugation problem	Ensure that centrifuge is in proper working condition
		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 × *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
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

23225 **BCA Protein Assay Kit**

23235 **Micro BCA™ Protein Assay Kit**

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