Zeba™ Spin Desalting Columns and Plates, 40K MWCO

Catalog Numbers A57756, A57758, A57759, A57760, A57761, A57762, A57763, A57764, A57765, A57766, A57767, and A57768

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Thermo Scientific™ Zeba™ Spin Desalting Columns and Plates, 40K molecular-weight cutoff (MWCO) use 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 <2,000 Da. Various spin-column volumes are available for processing samples ranging from 5–4,000 µL (Table 1). The spin-column and plate formats provide an excellent and optimal method for processing while preventing sample dilution typically associated with gravity-flow methods.

Contents and storage

Item	Amount	Catalog Number	Storage
Zeba™Spin Desalting Columns, Micro (75 µL)	25 columns	A57756	Store at room temperature.
	50 columns	A57758	
Zeba™ Spin Desalting Columns, 0.5 mL	25 columns	A57759	
	50 columns	A57760	
Zeba™ Spin Desalting Columns, 2 mL	5 columns	A57761	
	25 columns	A57762	
Zeba™ Spin Desalting Columns, 5 mL	5 columns	A57763	
	25 columns	A57764	
Zeba™ Spin Desalting Columns, 10 mL	5 columns	A57765	
	25 columns	A57766	
Zeba™ Spin Desalting Plates, 96-well	2 plates	A57767	
	4 plates	A57768	

Note: Products are recommended for processing compounds >40,000 Da. The resin slurry is supplied in 0.05% sodium azide.

Required materials not supplied

- For 75 µL and 0.5 mL spin columns:
  - Bench-top microcentrifuge capable of 700 x *g* (such as the Sorvall™ Legend™ Micro 17 Microcentrifuge)
  - 1.5 mL microcentrifuge tubes
- For 2- and 5-mL spin columns: and
  - Centrifuge capable of 700 x *g*
  - 15 mL conical tubes
- For 10 mL spin columns:
  - Centrifuge capable of 700 x *g*
  - 50 mL conical tubes
- For desalting plates: Variable-speed centrifuge with rotor and carrier capable of handling stacked plates (4.4 cm height) and 700 x *g*
- Wash/equilibration buffer

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

Additional 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 molecules removed and protein recovered are affected by the nature of the molecules and sample volume. The sample volumes recommended provide exceptional removal of various small molecules (typically >95% for molecules 1,500–2,000 Da); however, proteins and small molecules often behave differently than predicted because of various factors such as hydrophobicity, secondary structure, and interactions. Therefore, some optimization of sample volume might be needed 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 <1,000 Da and recovery of proteins and other macromolecules >7,000 Da.

Remove salt from proteins

For maximum protein recovery of low volumes, 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 the 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 flowthrough and replace the column back into the collection tube.
4. Add wash/equilibration buffer on top of the resin. Centrifuge tube and discard flowthrough. Repeat this step twice.

   Note: After each spin, the resin should appear white and free of liquid. If liquid is present, ensure the correct centrifugation speed and time. Incomplete centrifugation can result in poor sample recovery or sample dilution.
5. Blot the bottom of the column or plate to remove excess liquid. Transfer the column to a new collection tube or place the 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 flowthrough that contains sample. Discard the spin column.

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

		75 µL column	0.5 mL column	2 mL column	5 mL column	10 mL column	Plate
Sample volume range		5–14 µL	50–150 µL	300–800 µL	500–2,000 µL	1,000–4,000 µL	20–100 µL
Wash/equilibration buffer volume		50 µL	300 µL	1 mL	2.5 mL	5 mL	250 µL
Sample volume		<5 µL	<70 µL	<350 µL	<750 µL	<1,500 µL	<30 µL
Optional stacker volume		3 µL	15 µL	40 µL	100 µL	200 µL	10 µL
Centrifuge speed for all columns and plates		700 x *g*					
Centrifugation time	**Storage solution removal**	1 minute	1 minute	2 minutes	2 minutes	2 minutes	2 minutes
	Wash 1	1 minute	1 minute	2 minutes	2 minutes	2 minutes	2 minutes
	Wash 2	1 minute	1 minute	2 minutes	3 minutes	3 minutes	2 minutes
	Wash 3	1 minute	2 minutes	3 minutes	4 minutes	5 minutes	3 minutes
	Sample recovery	2 minutes	2 minutes	3 minutes	3 minutes	4 minutes	3 minutes

Troubleshooting

Observation	Possible cause	Recommended action
Sample or buffer does not flow through resin	Centrifugation problem.	Ensure centrifuge is working properly.
		Ensure bottom closure is removed.
		Ensure top cap is loosened.
Sample is contaminated	Sample was not properly loaded.	Apply sample directly to the center of the resin bed; touch tip to resin to expel all sample.
		Avoid contact with sides of column.
	Sample was not properly centrifuged.	For fixed-angle rotors, place the column in the same orientation each time and do not exceed the recommended centrifuge speed.
		Do not exceed the recommended centrifugation speed or time.
Low yield	Sample was not completely in solution before adding it to the column.	Centrifuge sample at 14,000 x g for 10 minutes before adding it to the column.
	Portion of protein remained in spin column.	Use a stacker to recover more protein (for most samples, most 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 sample, ensure the wash/equilibration buffer was adequately removed by centrifugation (that is, the column appears uniformly white with no solvent streaks).

Related products

Product	Cat. No.
Pierce™ BCA Protein Assay Kit	23225
Micro BCA™ Protein Assay Kit	23235
Pierce™ Rapid Gold BCA Protein Assay Kit	A53225
Pierce™ Dye and Biotin Removal Resin	A44296
Zeba™ Spin Desalting Columns, 7K MWCO	89882

Limited product warranty

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Thermo Fisher Scientific | 3747 N. Meridian Road | Rockford, Illinois 61101 USA

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision	Date	Description
A.0	10 August 2023	New document for Zeba™ Spin Desalting Columns and Plates, 40K MWCO.