INSTRUCTIONS

Polyacrylamide Desalting Columns

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43426

Number	Description
43426	**Polyacrylamide Desalting Columns**, 5 × 5mL columns, 5 porous discs, and a resin separator

Storage: Upon receipt store at 4°C. Do not freeze. Product shipped at ambient temperature.

Introduction

The Thermo Scientific Polyacrylamide Desalting Columns are ready-to-use, disposable desalting columns. Molecules greater than 1800 MW emerge in the void volume, separating them from the smaller molecules. A buffer exchange can also be accomplished by first equilibrating the column with a desired buffer and then applying the sample. Molecules greater than 1800 MW will emerge in the desired buffer.

These columns display excellent chromatographic properties and have an exclusion range from 100-1800 daltons. The Polyacrylamide 1800 gel is compatible with dilute organic acids, 8M urea, 6M guanidine•HCl, chaotropic agents, reducing agents and detergents. Up to 20% alcohol is compatible with the gel. Formamide is compatible with the column and may actually enhance the separation of complex mixtures of poorly water-soluble molecules. This column can be used with ultrapure water, but optimal separations are achieved using buffers containing ≥ 50mM of salt.

Inserting a porous disc into the top of each column provides a stop-flow function that prevents drying of the resin and loss of samples. Additional characteristics of the Polyacrylamide Desalting Columns are listed in Table 1.

Table 1. Characteristics of the 5mL Polyacrylamide Desalting Columns.

Characteristic	5mL column
Column volume (volume of the gel bed)	5mL
Void volume (~1/3 column volume)	1.75mL
Exclusion limit (for globular proteins)	1800Da
Column dimensions	90mm (H) x 12mm (D)
Gel particle size	45-90µm
pH range	2-10
Pressure resistance	15psi
Temperature range	4-80°C

Procedure for Buffer Exchange/Desalting

1. Invert column several times to resuspend the resin. Position the column upright in a test tube or clamp and allow the resin to settle for several minutes.
2. Remove the top cap from the column. Carefully pipette the storage solution (contains 0.02% azide) until 5-10mm of solution remains above the resin bed.
3. (Optional) Using the open end of the supplied resin separator, insert and slide a porous disc to within 1 mm of the resin bed. A top disc provides a stop-flow function that prevents disturbance and drying of the resin bed during use.
4. Twist off the removable closure on the bottom of the column. SAVE the bottom closure for later steps.
5. Equilibrate the column by adding five resin-bed volumes of buffer to the column and allowing it to drain through. Use a buffer into which you plan to exchange the sample.
6. Using a new collection tube, add the sample. For best results, use a sample volume ≤ 10% of the column resin-bed volume (e.g., 0.5mL for a 5mL column). Sample sizes up to 25% of the column volume may be appropriate in some circumstance, although the separation might not be optimal.
7. Allow the sample to enter the resin bed. A volume of equilibration buffer equal to the sample volume will emerge from the column.
8. Using a new collection tube, add a volume of buffer equal to the fraction volume you wish to collect (e.g., 0.5-1mL).
9. Allow the buffer to enter the resin bed and collect the buffer that emerges from the column.
10. Repeat steps 8 and 9 until the protein has emerged from the column.

    Note: Sample emergence can be monitored by measuring the absorbance of each fraction at 280nm. Generally, the first absorbance peak will emerge upon addition of one void volume* of buffer after the sample. This peak is the protein. Molecules smaller than the exclusion limit of the resin (e.g., buffer salts) will emerge from the column in subsequent fractions. These fractions can be discarded after confirming that all fractions containing protein have been collected.

    *The void volume is approximately 1/3 the resin-bed volume (See Table 1 on previous page).

11. Desalting columns can be regenerated by washing with 10 column volumes of buffer. For storage, wash the column with 5 resin-bed volumes of ultrapure water containing 0.02% sodium azide. When approximately 3 mL of solution remains above the resin bed, reseal the column by inverting the original snap-off closure, and with a slight twisting motion, press it firmly to the bottom tip of the column. Next, reseal to the top of the column with the original cap. Store the column at 4°C.

Related Thermo Scientific Products

89889	**Zeba™ Spin Desalting Columns, 5K MWCO, 2mL,** 5/pkg
89891	**Zeba Spin Desalting Columns, 5K MWCO, 5mL,** 5/pkg
43230	**Dextran Desalting Columns, 5K MWCO, 5mL,** 5/pkg
43233	**Dextran Desalting Columns, 5K MWCO, 10mL,** 5/pkg
68700	**SnakeSkin™ Dialysis Tubing, 7K MWCO,** 22mm dry I.D. × 35 feet

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition. The information in this guide is subject to change without notice.

Revision history: Pub. No. MAN0011198 B

Revision	Date	Description
B	31 July 2024	Correcting spin column usage.
A	17 October 2015	New document for Polyacrylamide Desalting Columns.