INSTRUCTIONS

Polyacrylamide Desalting Columns

43240 43243

0112.3

Number	Description
43240	**Polyacrylamide Desalting Columns,** 5 × 5mL
43243	**Polyacrylamide Desalting Columns,** 5 × 10mL

Note: Each package contains columns (5), column extenders (5), bottom plugs (5), porous discs (6) and a porous disc insertion tool.

Storage: Upon receipt store at room temperature or 4°C. Product shipped at ambient temperature.

Introduction

The Thermo Scientific Polyacrylamide Desalting Columns are gravity-flow devices for buffer exchanging or desalting protein samples > 6kDa. The columns contain porous, beaded polyacrylamide, which displays excellent chromatographic properties and sample recoveries. High flow rates and excellent resolution make this product ideal for both laboratory and industrial desalting.

The flow rates obtained with the Polyacrylamide Desalting Columns are up to 10 times greater than those obtained with other desalting columns. The 5mL Polyacrylamide Desalting Column has a flow rate of approximately 150mL/hour. The desalting process or buffer exchange can therefore be performed in less than 10 minutes.

Polyacrylamide is not subject to enzyme degradation and is resistant to microbial growth. The hydrophilic resin is essentially free of charge, which minimizes nonspecific binding. This resin is susceptible to hydrolysis of amide groups under extreme pH conditions; therefore use the columns at pH 2-10 at room temperature.

The Polyacrylamide Desalting Columns are compatible with a wide variety of buffers. The desalting resin is compatible with chaotropic agents such as 6M guanidine•HCl and 8M urea and solutions containing miscible organic solvents. These columns display unique stop-flow characteristics, which prevents the resin from drying and loss of valuable samples. Additional characteristics of the Polyacrylamide Desalting Columns are listed in Table 1.

Table 1. Characteristics of the Thermo Scientific Polyacrylamide Desalting Columns.

Characteristic	5mL Column	10mL Column
Resin Volume	5mL	10mL
Void Volume (~1/3 column volume)	1.75mL	3.5mL
Exclusion Limit (for globular proteins)	6000Da	6000Da
Wet Bead Diameter	90-180µm	90-180µm

Important Product Information

- The polyacrylamide resin is autoclavable at pH 5.5-6.5 in a suitable buffer (e.g., 0.05M citrate or HEPES) at 120°C for 15-30 minutes. Remove the polyacrylamide resin from the column before autoclaving.
- Periodically, the columns might require reconditioning to maintain their chromatographic properties. During use or storage, small air bubbles can form between the two discs that bracket the resin. Entrapped air bubbles in the resin bed can be removed by centrifugation for 10 minutes at 1000 × *g*. A centrifuge equipped with a swinging bucket rotor is optimal. Make sure to keep the top and bottom capped on during centrifugation. For optimal reconditioning, make sure there is buffer present above the top frit. A column might require two centrifugations to remove all of the entrapped air bubbles.

Procedure for Buffer Exchange/Desalting

1. Invert column several times to suspend 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 and 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 porous disc insertion tool, insert and slide a porous disc to within 1mm of the resin bed. A top porous disc provides a stop-flow function that prevents disturbance and drying of the resin during use.
4. Twist off bottom end tab.
5. Add five resin-bed volumes of buffer to the column and allow it to drain through. Use the buffer into which you plan to exchange the sample.
6. Place the column in or over a new collection tube and 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 cases, although the separation (i.e., desalting/buffer exchange) may not be optimal.
7. Allow the sample to enter the resin. A volume of buffer equal to the sample volume will drip from the column.
8. Place column over a new collection tube and add a volume of buffer equal to the fraction volume you wish to collect (e.g., 0.5-1mL). Allow the buffer fraction to enter the resin bed and collect the buffer that emerges from the column. Repeat this step until the protein has emerged from the column.

   Note: Protein emergence can be monitored by measuring the absorbance of each fraction at 280nm. The first absorbance peak (i.e., protein) will generally emerge when one void volume of buffer has been added after applying the sample. Molecules smaller than the resin's exclusion limit (e.g. buffer salts) will emerge from the column in subsequent fractions, which may be discarded after confirming that all protein-containing fractions have been collected.
9. Columns can be regenerated by washing with 10 column volumes of buffer. For storage, wash column with five column volumes of ultrapure water containing 0.02% sodium azide and a minimum of 0.05M NaCl. Cap the bottom then the top of the column when approximately 3mL of solution remains above the desalting resin.
10. Store the columns at 4°C.

    Note: To prevent resin swelling, always use buffer or ultrapure water containing at least 0.15M NaCl when equilibrating, washing and regenerating the resin. Keep the salt concentration consistent during equilibration, washing and regeneration.

Related Thermo Scientific Products

20439	**Excellulose Desalting Columns,** 5K MWCO, 5 × 2mL
20449	**Excellulose Desalting Columns,** 5K MWCO, 5 × 5mL
43230	**Dextran Desalting Columns,** 5K MWCO, 5 × 5mL
43233	**Dextran Desalting Columns,** 5K MWCO, 5 × 10mL
68700	**SnakeSkin® Dialysis Tubing,** 7K MWCO, 2mm dry I.D. × 35 feet

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

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