

Pierce[®] Chromatography Cartridges

Desalting

89934 89935

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Number	Description
89934	Pierce Chromatography Cartridge Desalting , 5 × 1 ml columns for 0.05-0.25 ml samples
89935	Pierce Chromatography Cartridge Desalting , 5 × 5 ml columns for 0.1-1.5 ml samples
	Note: Cartridges are for processing compounds > 7,000 Da. Resin is supplied in 0.05% sodium azide in sterile water. Each column pack contains one accessory pack (1 female Luer-Lok [®] Adapter, 1 connector fitting, 1 column plug and 5 bottom caps).
	Storage: Upon receipt store at 4-8°C. Product is shipped at ambient temperature. Do not freeze columns.

Introduction

The Pierce Desalting Cartridges contain Zeba[™] High-Performance Resin for desalting or buffer exchange applications. This resin is ideal for removing low molecular weight compounds including salts, fluorescent dyes, biotin and other small labeling reagents. Protein samples can be processed with unsurpassed protein recovery and ≥ 95% retention of salts and other small molecular contaminants (< 1,000 Da).

These cartridges are convenient, ready-to-use devices compatible with the major automated liquid-chromatography systems or for manual syringe processing (see Table 1 for general properties of the cartridges). The cartridges attach directly to ÄKTA[™] or FPLC Systems without additional connectors. An accessory pack included with each product readily adapts cartridges for use with Luer Syringe Fittings or 1/16" tubing. Pierce Desalting Cartridges provide fast, easy and reproducible chromatographic separations and can be regenerated for multiple uses.

Table 1. Properties of the Pierce Chromatography Cartridge Desalting.

Exclusion Limit	7,000 Da
Cartridge Dimensions	0.7 × 2.7 cm (1 ml column) 1.3 × 3.8 cm (5 ml column)
Void Volume	0.32 ml (1 ml column) 1.5 ml (5 ml column)
Recommended Flow Rate	0.2 -1.0 ml/min (1 ml column) 1-5 ml/min (5 ml column)
Maximum Recommended Flow Rate*	3 ml/min (1 ml column) 8 ml/min (5 ml column)
pH Limits	3-10
Maximum Operating Pressure	0.3 MPa, 43.5 psi or 3 bar
Cartridge Material	Polypropylene
Frit	Polyethylene, 10 µm
Storage Solution	0.02-0.05% Sodium azide
Accessory Pack	Luer-Lok Adapter to 10-32 male Finger-tight 10-32 connector fitting for 1/16" OD tubing Column plug for 10-32 coned port Cap 1/16 male

*At room temperature using water.

Important Product Information

- Use high purity buffers prepared with high-quality water. For best results, degas or filter buffers through a 0.45 µm filter.
- The resin is stable in solutions containing mild oxidants/reductants, salts, organic solvents, and alkaline or acidic conditions (pH 3-10).
- Do not freeze the resin as this will cause irreversible damage to the bead structure.
- Resin bed may shrink slightly in high ionic solutions, and slight compression of resin bed may occur temporarily with high operating pressure; however, these conditions will not affect performance.
- Pierce Cartridges may be used singly or connected in series (2-3 columns) to increase capacity. Back pressure will be greater when columns are used in a series than when used as single columns.
- To monitor protein as it emerges from the column, measure the UV absorbance at 280 nm. A highly concentrated sample results in a curve with a narrow absorbance peak.
- Sample resolution is partially controlled by the flow rate. For high resolution, use a slow flow rate (see LC procedure for suggested flow rates). When possible, monitor the unwanted low molecular weight compounds by conductivity or other means to evaluate resolution and to avoid pooling contaminant-containing fractions with the fractions that contain the target molecule.

Additional Materials Required

- Suitable liquid chromatography (LC) system (LC procedure only) with 1/16" tubing
- Syringes (syringe procedure only)
- Three-way Luer-Lok Stopcock may be used for the syringe processing (optional)
- Additional connectors and fittings are required to attach to the Bio-Rad BioLogic™ System

Procedure for Desalting or Buffer Exchange by Liquid Chromatography

1. Equilibrate the cartridge and all solutions to room temperature. Ensure all solutions are degassed.
2. Prepare the LC system by filling tubing with water or buffer. Remove top plug from cartridge and carefully snap off the end-tab (do not twist). To avoid introducing air into the system, let a few drops of buffer flow from tubing into column top then connect the column top to the tubing. Allow a few drops to emerge from the column before connecting to the LC inlet port.
3. Equilibrate the resin with 5-10 column volumes of water or buffer at a flow rate of 1 ml/minute for the 1 ml column or 5 ml/minute for the 5 ml column.
4. Apply the sample to the column. For best results, apply 0.05-0.25 ml samples for the 1 ml columns or 0.1-1.5 ml samples for the 5 ml columns. If the sample contains insoluble matter, centrifuge or filter (0.45 µm filter) it before applying to the column.

Note: For optimal separation when applying large molecules, such as proteins, use a slow flow rate (e.g., 0.2-1 ml/minute for the 1 ml columns or 0.5-2 ml/minute for the 5 ml columns).

5. Monitor protein and the low molecular weight compounds while collecting fractions (e.g., 0.1-0.5 ml for 1 ml columns or 0.5-1 ml for 5 ml columns).
6. Before applying another sample, wash the resin with water or buffer until the absorbance at 280 nm approaches baseline (~3-5 column volumes). Cartridge may be reused multiple times without significant loss of performance.
7. For storage, wash the resin with five column volumes of 0.02-0.05% sodium azide in water. Attach supplied bottom cap followed by the top plug. Store cartridge at 4°C.

Procedure for Desalting or Buffer Exchange using a Syringe

Note: The void volumes are 0.320 ml for the 1 ml columns and 1.5 ml for the 5 ml columns.

1. Equilibrate the cartridge and all solutions to room temperature. Ensure all solutions are degassed.
2. Fill a syringe with 5-10 column volumes of water or buffer.
3. Attach the syringe to the Luer-Lok Adapter included in the accessory pack. Remove top plug from column and carefully snap off the end-tab. To avoid introducing air into the system, allow a few drops to emerge from the Luer-Lok Adapter and then connect to the column top. Securely tighten the connection.
4. Equilibrate the resin with 5-10 column volumes of water or buffer at a flow rate of ~1 ml/minute for the 1 ml column or ~5 ml/minute for 5 ml column. Remove syringe from the Luer-Lok Adapter.
5. Fill an appropriately sized syringe with the sample and connect it to the Luer-Lok Adapter. For best results, use 0.05-0.25 ml samples for the 1 ml column or 0.1-1.5 ml samples for the 5 ml column. If the sample contains insoluble matter, centrifuge or filter (0.45 µm filter) it before applying to the column.
6. Apply the sample to the column at a controlled flow rate of 0.2-1 ml/minute for the 1 ml column or 0.5-2 ml/minute for the 5 ml column. As soon as the syringe is empty, remove it from the Luer-Lok Adapter.
7. Fill an appropriately sized syringe with water or buffer and connect it to the Luer-Lok Adapter. Depress the syringe plunger to pass the sample through the column.
8. Collect fractions containing the high molecular weight compounds, which emerge after one void volume has passed through the column. The void volumes for the 1 ml and 5 ml columns are 0.320 ml and 1.5 ml, respectively. The compounds of interest generally emerge from 0.3 to 0.9 ml for the 1 ml columns or 1.5 to 4.5 ml for the 5 ml columns.
9. Wash the resin with 3-5 column volumes of water or buffer before applying the next sample. Cartridge may be reused multiple times without significant loss of performance.
10. For storage, wash resin with five column volumes of 0.02-0.05% sodium azide in water. Attach the supplied bottom cap and then the top plug. Store cartridge at 4°C.

Troubleshooting

Problem	Possible Cause	Solution
Absorbance peak is too broad	Using sample loops can sometimes result in broad peaks	Inject sample onto column using direct syringe loading rather than a sample loop
Poor separation of peaks	Flow rate is too fast	Decrease flow rate especially during sample application
	High sample viscosity relative to desalting buffer	Dilute viscous protein samples before applying to the column

Related Thermo Scientific Products

- 28372** **BupH™ Phosphate Buffered Saline Packs, 40 packs**
- 28376** **BupH Tris Buffered Saline Packs, 40 packs**
- 28384** **BupH Borate Buffer Packs, 40 packs**
- 28382** **BupH Carbonate-Bicarbonate Buffer Packs, 40 packs**

Current versions of product instructions are available at www.piercenet.com. For a faxed copy, call 800-874-3723 or contact your local distributor. Luer-Lok® is a trademark of Becton-Dickinson.

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