

HiTrap Desalting Column

Product Specification

HiTrap® Desalting Columns apply size-exclusion chromatography to facilitate removal of low molecular weight components (MW<1000Da) from DNA, proteins, or peptides (MW>5000Da). These 5mL columns are packed with Sephadex® G-25 Superfine media and are shipped ready for installation into an FPLC® system or use with a syringe. To connect these columns to an HPLC system, simply use one of the three unions listed in the ordering information.

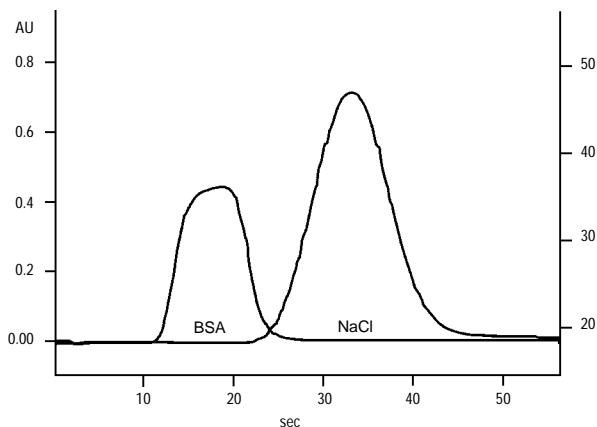
Separation of 0.2-1.5mL of sample can be achieved in less than 30 seconds with flow rates up to 15mL/min (Figure A). The sample volume can easily be increased to 7.5mL by connecting up to 5 columns in series (Figure B). The use of more than one HiTrap Desalting Column can provide better resolution of a given sample (Figure C). Negligible, nonspecific interactions are seen even at very low (5mM) buffer concentrations, allowing for protein recoveries of >95%. Typical characteristics of HiTrap Desalting Columns are presented in Table 1.

Desalting, or buffer exchange, is a common operation for the following:

- sample preparation — before or after labeling, electrophoresis, enzyme assays, or other chromatography modes
- stopping reactions between macromolecules and low molecular weight reagents
- prior to sample storage
- after chemical oxidation/reduction

Figure A. Highly Efficient Desalting in Half a Minute

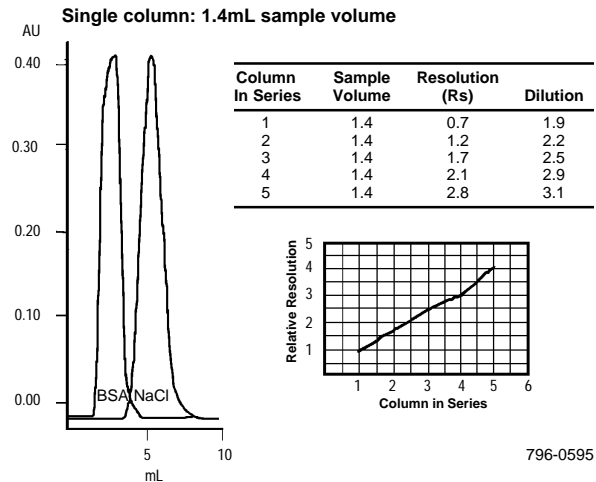
Column: **HiTrap Desalting Column, 250 x 16mm ID**
 Cat. No.: **54822**
 Mobile Phase: 0.05M NaPO₄, 0.15M NaCl, pH 7.0
 Flow Rate: 10mL/min (6sec/mL)
 Det.: UV, 280nm, and conductivity
 Inj.: 1.4mL of 2mg BSA/mL in 0.5M NaCl, 0.05M NaPO₄, pH 7.0
 System: FPLC



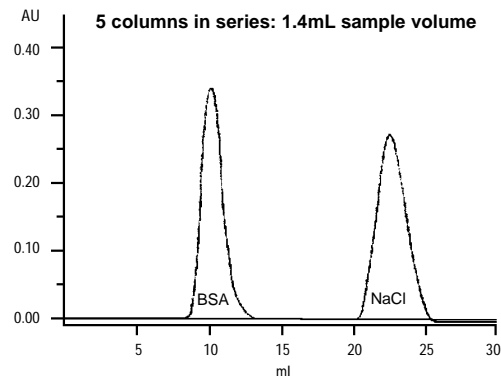
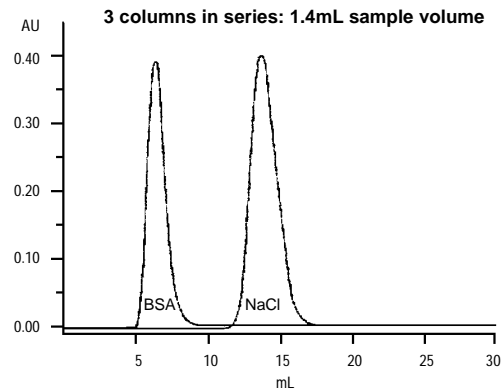
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Figure B. HiTrap Desalting Columns Coupled in Series, 1.4mL Sample Volume

Column: **HiTrap Desalting Column, 250 x 16mm ID**
 Cat. No.: **54822**
 Mobile Phase: 0.05M NaPO₄, 0.15M NaCl, pH 7.0
 Flow Rate: 5mL/min
 Det.: UV, 280nm, and conductivity
 Inj.: 1.4mL of 2mg BSA/mL in 0.5M NaCl, 0.05M NaPO₄, pH 7.0
 System: FPLC



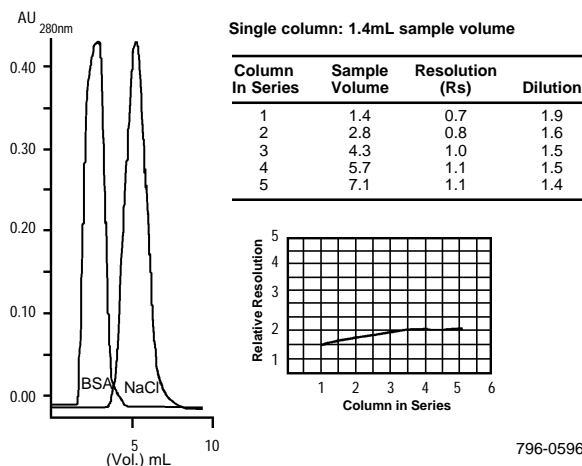
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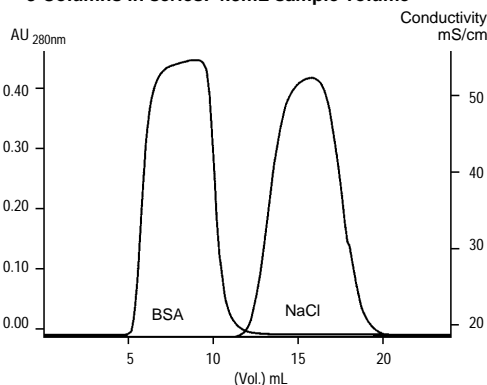
Figure C. HiTrap Desalting Columns Coupled in Series, Increasing Sample Volume

Column: **HiTrap Desalting Column, 250 x 16mm ID**
 Cat. No.: **54822**
 Mobile Phase: 0.05M NaPO₄, 0.15M NaCl, pH 7.0
 Flow Rate: 5mL/min
 Det.: UV, 280nm, and conductivity
 Inj.: 28% of total column volume, 2mg BSA/mL in 0.5M NaCl, 0.05M NaPO₄, pH 7.0
 System: FPLC

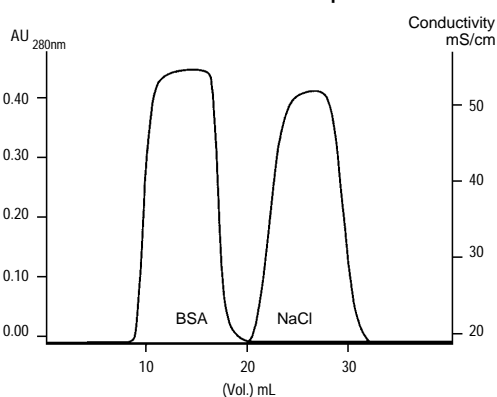


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3 Columns in series: 4.3mL sample volume



5 Columns in series: 7.1mL sample volume



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Table 1. Characteristics of HiTrap Desalting Columns

Property	HiTrap Desalting Column
Separation mechanism	Size exclusion
Bed volume	5mL
Bed dimension	1.6 x 2.5cm
Void volume	1.5mL
Recommended sample volume	0.1-1.5mL
Matrix	Sephadex G-25 Superfine
Bead size	15-70µm
Exclusion limit	5000Da
Maximum flow rate [■]	15mL/min
Recommended flow rate [■]	1-10mL/min
Backpressure at 10mL/min [■]	0.25bar
Pressure limit ^{■ ■}	3bar, 44psi, 0.3MPa
Chemical stability	All commonly used buffers
pH stability, short and long term ^{■ ■ ■}	2-13
Storage	20% ethanol
Avoid	Oxidizing agents

- Room temperature, aqueous buffers
- Maximum pressure at zero flow rate without leakage
- Short term refers to the pH interval for regeneration. Long term refers to the pH interval where the gel is stable over a long period of time, without adverse effects on its subsequent chromatographic performance.

Ordering Information:

Description	Cat. No.
HiTrap Desalting Columns, 5mL, pk. of 5	54822
Unions, 1/16" to M6, female, pk. of 2	
Waters	54866
Swagelok	54867
Valco	54868

Trademarks

FPLC, HiTrap, Sephadex — Pharmacia Biotech AB

Acknowledgments

The figures were provided by Pharmacia Biotech AB.

Contact our Technical Service Department (phone 800-359-3041 or 814-359-3041, FAX 814-359-5468) for expert answers to your questions.

For more information, or current prices, contact your nearest Supelco subsidiary listed below. To obtain further contact information, visit our website (www.sigma-aldrich.com), see the Supelco catalog, or contact Supelco, Bellefonte, PA 16823-0048 USA.

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