HiTrap Desalting
HiPrep 26/10 Desalting

Introduction

HiTrap™ Desalting is a prepacked, ready to use 5 ml column for fast and convenient group separations between high and low molecular weight substances (Fig 1).

HiPrep™ 26/10 Desalting is a prepacked ready to use 53-ml column for larger sample volumes (Fig 2).

Since proteins and other biomolecules differ greatly in size from salts and other small molecules, medium filtration is particularly efficient for many everyday laboratory operations. "Group separation" separates the components of a sample into two groups, e.g. high molecular weight substances, which are excluded from the medium and thus elute first, from low molecular weight substances that enter the pores freely and thus elute later.

Buffer exchange and desalting are common operations in any laboratory engaged in sample purification and analysis. It is often necessary to change the buffer composition of a sample between chromatography steps or to satisfy special requirements of an assay. Desalting of a sample is a prerequisite for mass spectroscopy analysis, lyophilization, and after certain procedures such as ion exchange chromatography.

Although both procedures can be accomplished by dialysis, this is a time-consuming process, and samples sensitive to degradation may be at risk. Because of its high speed and high volume capacity, HiPrep 26/10 Desalting is an excellent alternative to dialysis, especially when larger sample volumes are used or when samples need to be processed rapidly to avoid degradation.

The fractionation range for globular proteins is between M_r 1 000–5 000, with an exclusion limit of approximately M_r 5 000. This ensures group separations of proteins/peptides larger then M_r 5 000 from molecules with a molecular weight less than M_r 1 000. Separations are easily performed with a syringe (only HiTrap), peristaltic pump, or chromatography system, such as ÄKTAdesign™.

Fig 1. HiTrap Desalting gives fast and convenient group separations between high and low molecular weight substances.

Fig 2. HiPrep 26/10 Desalting for fast desalting and buffer exchange.

HiTrap Desalting and HiPrep Desalting have the following advantages:

- Replaces dialysis.
- Fast and convenient use.
- High recovery, reliable and reproducible separations.
- Easy scale up of sample volumes by connecting up to five columns in series.
- Well-suited to work where columns must be discarded after use.

Sephadex

HiTrap Desalting

Medium characteristics

HiTrap Desalting is packed with the well-known gel filtration medium Sephadex™ G-25 Superfine. The matrix is based on cross-linked dextran beads.

HiTrap Desalting can be used with aqueous solutions in the pH range 2–13. It is stable to all commonly used buffers, 8 M urea, 6 M guanidine hydrochloride, and all non-ionic and ionic detergents.

The recommended range of sample volume is 0.1–1.5 ml when complete removal of low molecular weight components is desired.

The separation is not affected by the flow rate in the range 1–15 ml/min. The maximum recommended flow rate is 15 ml/min. Table 1 shows the main characteristics of HiTrap Desalting.

Column characteristics

The column is made of biocompatible polypropylene with polyethylene frits. The column is delivered with a stopper on the inlet and a twist-off end on the outlet. The column cannot be opened or refilled.

Operation

Complete, easy-to-follow instructions are included in each pack of columns. Operation is easy, using either a syringe, an Eppendorf Multipipette™ (multi-dispensing pipette), peristaltic pump or liquid chromatography system. Larger sample volumes or, if required, higher resolution can easily be achieved by connecting up to five columns in series. See Table 2 for recommended sample and elution volumes.

A set of connectors is supplied for connecting the column to different equipments. HiTrap Desalting columns are delivered in 20% ethanol as bacteriostatic agent.

Applications

HiTrap Desalting can be used wherever removal of low molecular weight components is needed. Some examples are given below:

- Preparing samples prior to or after ion exchange, affinity or hydrophobic interaction chromatography.
- Removing free low molecular weight labels or contaminants from proteins/peptides.
- Removing substrates, inhibitors or co-factors from enzymes.
- Preparing samples for concentration, freeze-drying or storage.
- Terminating a reaction between a macromolecule and a low molecular weight reagent.

Table 1. Characteristics of HiTrap Desalting

Matrix	Sephadex G-25 Superfine, cross-linked dextran
Bed volume	5 ml
Bed dimension	1.6 × 2.5 cm
Void volume	1.5 ml
Recommended sample volume	0.1–1.5 ml
Sample dilution, syringe operation	1.3–4.0 fold
Exclusion limit	M_r 5 000, globular proteins
Average particle size	90 µm
Maximum flow rate*	15 ml/min
Recommended flow rate*	1–10 ml/min
Back pressure at 10 ml/min**	0.25 bar
Pressure limit**	3 bar, 44 psi, 0.3 MPa
Chemical stability	All commonly used buffers
pH stability, short and long term***	2–13
Avoid	Oxidizing agents
Storage	20% ethanol

* Room temperature, aqueous buffers.

** Maximum pressure at zero flow without leakage.

*** Short term refers to the pH interval for regeneration. Long term refers to the pH interval where the medium is stable over a long period of time without adverse effects on its performance.

Desalting in a fraction of a minute

Thanks to the rigidity of the Sephadex G-25 matrix and an optimized column packing method, HiTrap Desalting has a very low back pressure (only 0.25 bar, (3.7 psi) at 10 ml/min). This low back pressure makes it possible to run separations in a fraction of a minute using a syringe or a pump. Figure 3, a separation of BSA and NaCl, shows evidence of this.

The whole separation took only 45 seconds and the protein was eluted in less than 30 seconds.

When desalting samples, HiTrap Desalting gives the same efficient separation pattern independent of flow rate in the range 1–15 ml/min.

Table 2. Recommended sample and elution volumes using a syringe or a pipette. Examples of typical yields and remaining salt in the desalted material

Sample load ml	Add buffer ml	Elute and collect ml	Yield %	Remaing salt %	Dilution factor
0.25	1.25	1.0	>95	0.0	4.0
0.50	1.0	1.5	>95	<0.1	3.0
1.00	0.5	2.0	>95	>0.2	2.0
1.50	0	2.0	>95	>0.2	1.3

Column: HiTrap Desalting
Sample: 1.4 ml BSA (2 mg/ml) in 50 mM sodium phosphate, 0.5 M sodium chloride, pH 7.0
Buffer: 50 mM sodium phosphate, 0.15 M sodium chloride, pH 7.0
Flow rate: 10 ml/min

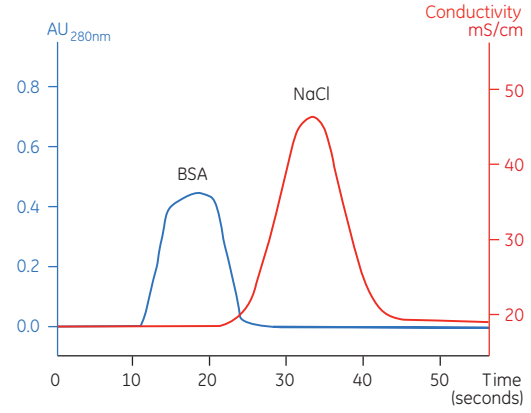

Fig 3. Highly efficient desalting in half a minute using HiTrap Desalting.

Removal of components following a coupling reaction using a syringe

Human IgG (1 ml, 18.3 mg) was coupled to the prepacked column, HiTrap NHS-activated HP (N-hydroxysuccinimide pre-activated medium). After 30 min reaction, unbound protein together with N-hydroxysuccinimide released during the coupling reaction was washed out with 3.8 ml coupling buffer and collected. Coupling yield was determined by separating 0.5 ml of the collected material on a HiTrap Desalting column, according to the recommendations in Table 2. The column was operated using a syringe and fractions of 0.5 ml were collected to visually follow the separation. Absorbance at 280 nm was measured (Fig 4). 1.9 mg human IgG was found in the washing solution giving a coupling yield of 89.7% (16.4 mg).

Column: HiTrap Desalting
Sample: 0.5 ml unbound human IgG in 0.2 M sodium carbonate, pH 8.5 after coupling to a HiTrap NHS-activated HP 1 ml column
Buffer: 0.2 M sodium carbonate, pH 8.5
Fractions: 0.5 ml

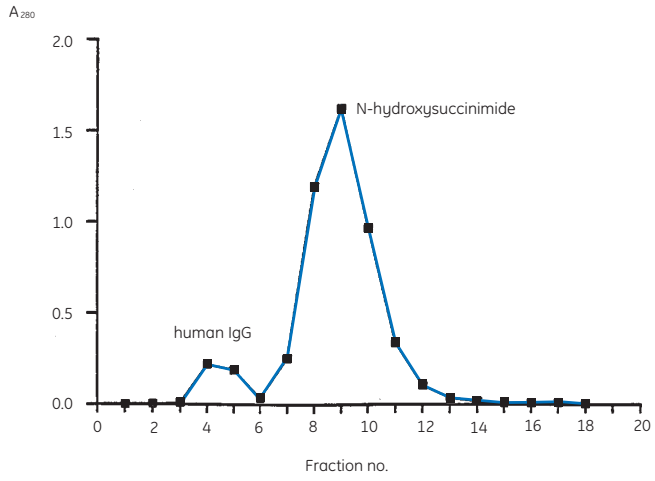

Fig 4. Removing components following a coupling reaction.

Scaling up by connecting HiTrap Desalting columns in series

Larger sample volumes or, if required, better resolution, can easily be achieved by connecting up to five columns in series. Figure 5 a–c show the results obtained when one, three and five HiTrap Desalting columns were connected in series. The sample volumes were 1.4, 4.3 and 7.1 ml respectively.

Column: HiTrap Desalting, 1 × 5 ml, 3 × 5 ml, 5 × 5 ml
Sample: 2 mg/ml BSA in 50 mM sodium phosphate, 0.5 M sodium chloride, pH 7.0
Sample vol.: 28% of column volume (1.4, 4.3 and 7.1 ml respectively)
Buffer: 50 mM sodium phosphate, 0.15 M sodium chloride, pH 7.0
Flow rate: 5 ml/min

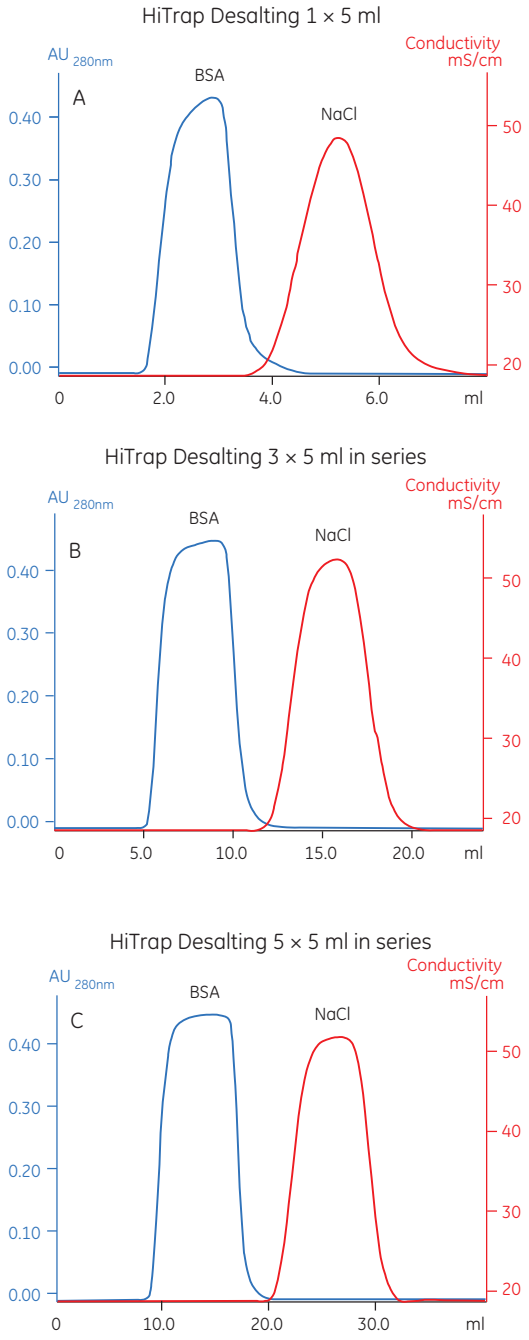

Fig 5. HiTrap columns connected in series.

On-line buffer exchange

HiTrap Desalting columns can easily be attached to other columns for on-line buffer exchange prior to or after a chromatographic step. An example is shown in Figure 6, where a HiTrap Desalting column is connected in series after a HiTrap Protein G HP column to adjust the pH of the eluted material. The HiTrap Desalting column was equilibrated with 20 mM sodium phosphate, pH 7.0.

A mouse monoclonal antibody (IgG_1) from a serum-free cell culture supernatant was purified using HiTrap Protein G HP 1 ml column. After sample application (5 ml) and washing with 5 ml binding buffer (20 mM sodium phosphate, pH 7.0), bound material was eluted with 5 ml 0.1 M glycine, pH 2.7. The first 1.3 ml eluted from the HiTrap Protein G HP column was discarded before the column was connected to the inlet of the HiTrap Desalting column. The eluate from the columns was monitored for UV absorbance and pH was measured on 0.5 ml collected fractions.

Figure 6a shows the original separation without the extra desalting step. The obtained yield was 18% higher when the HiTrap Desalting column was used for on-line desalting (Fig 6b).

Columns:	a) HiTrap Protein G HP, 1 ml b) HiTrap Desalting, 5 ml
Sample:	Eluted mouse monoclonal IgG from HiTrap Protein G HP
Binding buffer:	20 mM sodium phosphate, pH 7.0
Elution buffer:	0.1 M glycine, pH 2.7
Flow rate:	2 ml/min

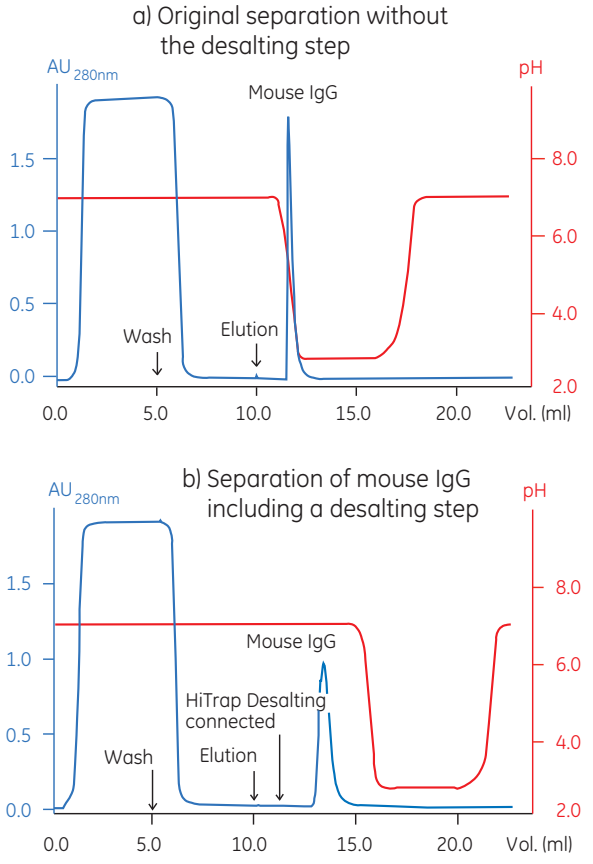

Fig 6. On-line buffer exchange with HiTrap Desalting improves yield on a mouse IgG purification.

HiPrep 26/10 Desalting

Medium characteristics

Sephadex G-25 Fine is prepared by cross-linking dextran with epichlorohydrin. The average particle size is 90 µm in 0.15 M NaCl.

The fractionation range for globular proteins is between M_r 1 000–5 000, with an exclusion limit of approximately M_r 5 000. This ensures group separations of proteins/peptides larger than 5 000 from molecules with a molecular weight less than 1 000.

Column characteristics

HiPrep 26/10 Desalting is packed with Sephadex G-25 Fine. The column has an internal diameter of 2.6 cm and a bed height of 10 cm. The medium bed volume is approximately 53 ml.

The HiPrep column is made of biocompatible polypropylene with nylon frits. The set of connectors supplied makes it easy to connect the column to different chromatography systems.

The column is not designed to be opened or repacked.

The characteristics of HiPrep 26/10 Desalting are summarized in Table 3.

Table 3. Characteristics of HiPrep 26/10 Desalting

Matrix	Sephadex G-25 Fine, cross-linked dextran
Bed volume	53 ml
Bed dimensions, i.d × h	2.6 × 10 cm
Void volume	15 ml
Recommended sample volume	≤ 15 ml
Sample dilution	1.2–3 fold
Exclusion limit	M_r 5 000, globular proteins
Average particle size	90 µm
Recommended flow rate*	9–31 ml/min (100–350 cm/h)
Maximum flow rate*	40 ml/min (450 cm/h)
Maximum pressure over the packed bed	0.15 MPa, 1.5 bar
Maximum pressure over column hardware	0.5 MPa, 5 bar
Chemical stability	All commonly used buffers
pH stability** short and long term	2–13
Avoid	Oxidizing agents
Storage	20% ethanol

* Water at room temperature

** Short term refers to the pH interval for regeneration. Long term refers to the pH interval where the medium is stable over a long period of time without adverse effects on its performance.

Parameters Affecting Desalting

The most critical parameter affecting resolution, R_S, in desalting applications is the sample-to-medium volume ratio. To minimize dilution and still retain good separation, sample volumes up to approximately 30% of the total bed volume are recommended. Figure 7 illustrates how the resolution (R_S) of a group separation run on HiPrep 26/10 Desalting is affected by sample volume.

Desalting can be performed at high flow rates as flow rate has a minor impact on resolution. This is illustrated in Figure 8.

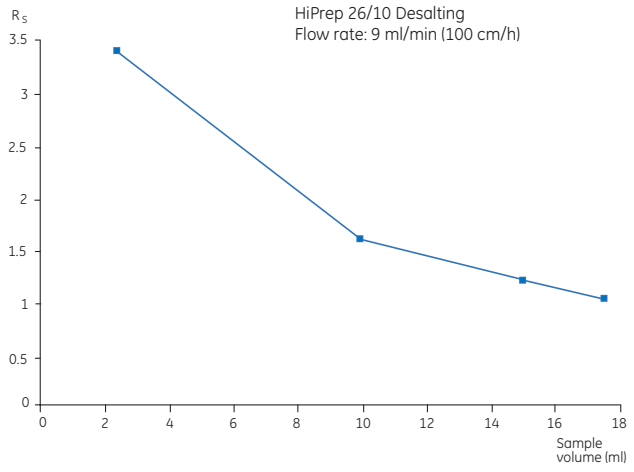

Fig 7. Influence of sample volume on the resolution.

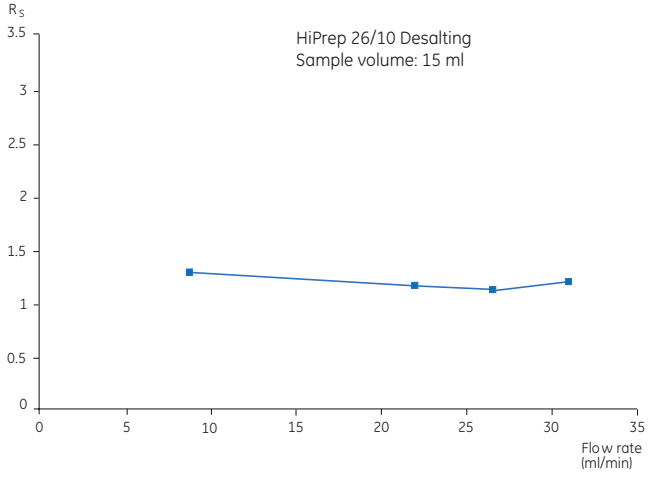

Fig 8. Influence of flow rate on the resolution.

Applications

Buffer exchange of mouse plasma

Mouse plasma was buffer exchanged within 1.5 minutes on HiPrep 26/10 Desalting. 10 ml of sample was applied at a flow rate of 22 ml/min (250 cm/h). Figure 9 shows the chromatogram of the separation. The protein was eluted in a volume of 19 ml.

Column:	HiPrep 26/10 Desalting
Sample:	Mouse plasma, centrifuged at 10 000 x g for 10 min
Sample volume:	10 ml
Buffer:	25 mM sodium acetate, pH 7.0
Flow rate:	22 ml/min (250 cm/h)
Instrumentation:	ÄKTAexplorer 100 with 1 mm i. d. tubing installed

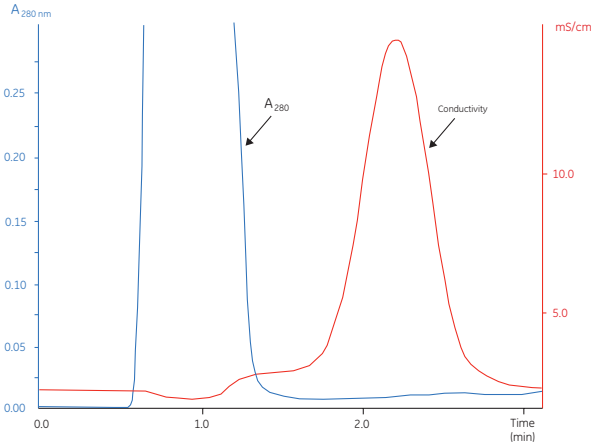

Fig 9. Buffer exchange of mouse plasma on HiPrep 26/10 Desalting.

Reproducible removal of N-Hydroxysuccinimide from bovine serum albumin

Figure 10 shows three runs on the removal of N-Hydroxysuccinimide (M_r 115) from bovine serum albumin (M_r 67 000). Highly efficient and reproducible desalting was achieved in all three runs.

Column:	HiPrep 26/10 Desalting
Sample:	2 mg/ml BSA, 0.07 mg/ml N-Hydroxysuccinimide (NHS) in 50 mM sodium phosphate, 0.15 M NaCl, pH 7.0. Filtered through a 0.45 µm filter
Sample volume:	13 ml
Buffer:	50 mM sodium phosphate, 0.15 M NaCl, pH 7.0
Flow rate:	31 ml/min (350 cm/h)
Instrumentation:	ÄKTAexplorer™ 100 (1 mm i. d. tubing installed)

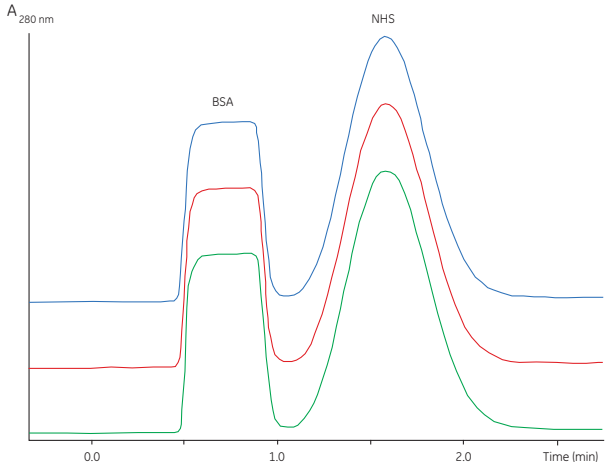

Fig 10. Reproducible removal of N-Hydroxysuccinimide from bovine serum albumin.

Scaling-up sample volumes

Samples of 15 ml (30% of the total bed volume) or less can be applied to a single column, and by coupling up to four columns in series, see Figure 11, a maximum sample volume of 60 ml can be run. Even with four columns in series, high flow rates can be maintained without back pressure problems, resulting in fast separations. In fact, in 20–30 min, up to 60 ml of sample can be desalted or buffer exchanged.

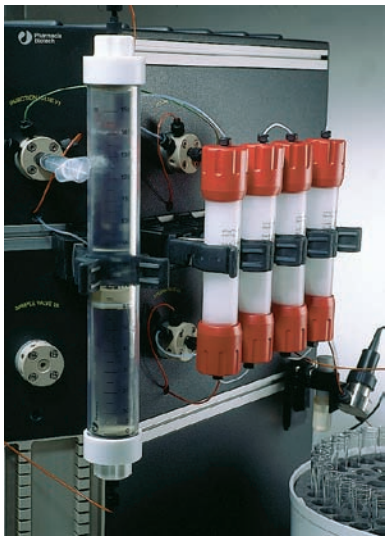

Fig 11. Four HiPrep 26/10 Desalting columns connected in series.

Table 4 shows how sample volumes can be scaled up using HiPrep 26/10 Desalting columns in series. Samples consisted of either 30 or 60 ml of a fungal culture supernatant, containing a secreted recombinant protein. Samples were run on two (30 ml sample) or four (60 ml sample) columns connected in series.

GE Healthcare offers a range of columns for desalting and buffer exchange that can accommodate variations in sample size and yield acceptable levels of dilution. These are summarized in Table 5.

Results

Sample loaded: 30 ml
Sample eluted: 35 ml (dilution factor, 1.2)

Sample loaded: 60 ml
Sample eluted: 70 ml (dilution factor, 1.2)

Table 4. Run data for buffer exchange of 30 ml and 60 ml samples on HiPrep 26/10 Desalting columns connected in series

Columns:	2 × HiPrep 26/10 Desalting in series (V_T = 106 ml) for 30 ml sample or 4 × HiPrep 26/10 Desalting in series (V_T = 212 ml) for 60 ml sample
Sample:	30 ml or 60 ml *Pichia pastoris* culture supernatant containing a secreted recombinant protein
Sample preparation:	Filter through 0.45 µm filter
Sample loop:	Superloop™ 150 ml
Buffer:	0.1 M Tris, 0.15 M NaCl, 0.05% Tween™ 20, pH 7.6
Flow rates (30 ml):	
sample loading	12 ml/min
elution	17 ml/min
Flow rates (60 ml):	
sample loading	8 ml/min
elution	11 ml/min
Back pressure:	0.48 MPa, 4.8 bar
Instrumentation:	ÄKTAexplorer 100

Table 5. Summary of desalting/buffer exchange columns

Column	Loaded volume (ml)	Eluted volume (ml)	Dilution factor	Operation
HiPrep 26/10 Desalting	10	10–15	1–1.5	pump
	15 (max)	15–20	1–1.3	pump
2 x HiPrep 26/10 Desalting	30 (max)	30–40	1–1.3	pump
3 x HiPrep 26/10 Desalting	45 (max)	45–55	1–1.2	pump
4 x HiPrep 26/10 Desalting	60 (max)	60–70	1–1.2	pump
HiTrap™ Desalting	0.25	1.0	4	syringe/pump
	0.5	1.5	3	syringe/pump
	1.0	2.0	2	syringe/pump
	1.5 (max)	2.0	1.3	syringe/pump
2 x HiTrap Desalting	3.0 (max)	4–5	1.3–1.7	syringe/pump
3 x HiTrap Desalting	4.5 (max)	6–7	1.3–1.7	syringe/pump
PD–10	1.5	3.5	2.3	gravity
	2.0	3.5	1.8	gravity
	2.5 (max)	3.5	1.4	gravity

Ordering information

Product	Quantity	Code No.
HiTrap Desalting	5 × 5 ml	17-1408-01
HiTrap Desalting	100 × 5 ml*	11-0003-29
HiPrep 26/10 Desalting	1 (53 ml)	17-5087-01
HiPrep 26/10 Desalting	4 (53 ml)	17-5087-02

* Special pack size delivered on specific customer order.

Related literature	Code No.
Gel Filtration Handbook, Methods and Principles	18-1022-18
Gel Filtration Column and Media, Selection Guide	18-1124-19
Convenient Protein Purification, HiTrap Column Guide	18-1129-81

Accessories	Quantity	Code No.
1/16" male/Luer female*	2	18-1112-51
Tubing connector flangeless/M6 female*	2	18-1003-68
Tubing connector flangeless/M6 male*	2	18-1017-98
Union 1/16" female/M6 male*	6	18-1112-57
Union M6 female /1/16" male	5	18-3858-01
Union Luerlock female/M6 female	2	18-1027-12
HiTrap/HiPrep, 1/16" male connector for ÄKTAdesign	8	28-4010-81
Stop plug female, 1/16"†	5	11-0004-64
Fingertight stop plug, 1/16"‡	5	11-0003-55

* One connector included in each HiTrap package

† Two, five, or seven female stop plugs included in HiTrap packages, depending on products

‡ One fingertight stop plug is connected to the top of each HiTrap column

www.gelifesciences.com/hitrap
www.gelifesciences.com/protein-purification

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

GE Healthcare Europe GmbH
Munzinger Strasse 5, D-79111 Freiburg, Germany

GE Healthcare UK Ltd
Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

GE Healthcare Bio-Sciences Corp
800 Centennial Avenue, P.O. Box 1327, Piscataway,
NJ 08855-1327, USA

GE Healthcare Bio-Sciences KK
Sanken Bldg. 3-25-1, Hyakunincho, Shinjuku-ku,
Tokyo 169-0073, Japan

Asia Pacific T +85 65 62751830 F +85 65 62751829 • Australasia T +61 2 8820 8299 F +61 2 8820 8200 • Austria T 01 /57606 1613 F 01 /57606 1614 • Belgium T 0800 73 890 F 02 416 8206 • Canada T 1 800 463 5800 F 1 800 567 1008 • Central & East Europe T +43 1 972 720 F +43 1 972 722 750 • Denmark T +45 70 25 24 50 F +45 45 16 2424 • Eire T 1 800 709992 F +44 1494 542010 • Finland & Baltics T +358 9 512 3940 F +358 9 512 39439 • France T 01 69 35 67 00 F 01 69 41 98 77 Germany T 0800 9080 711 F 0800 9080 712 • Greater China T +852 2100 6300 F +852 2100 6338 • Italy T 02 26001 320 F 02 26001 399 • Japan T 81 3 5331 9336 F 81 3 5331 9370 • Korea T 82 2 6201 3700 F 82 2 6201 3803 • Latin America T +55 11 3933 7300 F +55 11 3933 7304 • Middle East & Africa T +30 210 96 00 687 F +30 210 96 00 693 • Netherlands T 0800-82 82 82 1 F 0800-82 82 82 4 • Norway T +47 815 65 777 F +47 815 65 666 • Portugal T 21 417 7035 F 21 417 3184 • Russia, CIS & NIS T +7 495 956 5177 F +7 495 956 5176 • Spain T 902 11 72 65 F 935 94 49 65 • Sweden T 018 612 1900 F 018 612 1910 • Switzerland T 0848 8028 10 F 0848 8028 11 • UK T 0800 515 313 F 0800 616 927 • USA T +1 800 526 3593 F +1 877 295 8102

imagination at work

28-9137-87 AA 04/2007