GE Healthcare

Instructions 71-7154-00 AK **HiTrap Desalting columns**

HiTrap Desalting

HiTrap™ Desalting is a prepacked, ready to use column for group separation between high and low molecular weight substances, i.e., buffer exchange prior to or after different chromatographic steps, removal of low molecular weight contaminants or removal of reagents to terminate a reaction.

The special design of the column, together with the well-known media, Sephadex™ G-25 Superfine, provides fast, reproducible and easy separations in a convenient format. Separations are easily performed with a syringe, a pump or a chromatography system such as ÄKTAdesign™.

HiTrap

Code No.	Designation	No. supplied
17-1408-01	HiTrap Desalting	5 × 5 ml
11-0003-29	HiTrap Desalting	100 × 5 ml*

* Special pack delivered on specific customer order.

Connectorkit

Connectors supplied	Usage	No. supplied
1/16" male/luer female	Connection of syringe to top of HiTrap column	1
Tubing connector flangeless/M6 female	Connection of tubing (e.g. Peristaltic Pump P1) to bottom of HiTrap column*	1
Tubing connector flangeless/M6 male	Connection of tubing (e.g. Peristaltic Pump P1) to top of HiTrap column**	1
Union 1/16" female/ M6 male	Connection to original FPLC™ System through bottom of HiTrap column	1
Union M6 female/ 1/16" male	Connection to original FPLC System through top of HiTrap column	1
Stop plug female, 1/16"	Sealing bottom of HiTrap column	2, 5 or 7

* Union 1/16" female/M6 male is also needed.
** Union M6 female/1/16" male is also needed.

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1. Description

Medium properties

The HiTrap Desalting column is packed with the well-known size exclusion medium Sephadex G-25 Superfine. The medium is based on cross-linked dextran beads which allow excellent resolution and high flow rates. 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.

HiTrap Desalting can be used with aqueous solutions in the pH range 2–13. It is stable to all commonly used buffers, solutions of urea (8 M), guanidine hydrochloride (6 M), and all non-ionic and ionic detergents. Lower alcohols (methanol, ethanol, propanol) may be used in the buffer or the sample, but we recommend that the concentration be kept below 25 v/v%. Prolonged exposure (hours) to pH values below 2 or above 13, or to oxidizing agents should be avoided.

The recommended range of sample volumes 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 1–10 ml/min. The maximum recommended flow rate is 15 ml/min. Characteristics of the HiTrap Desalting column are summarized in Table 1.

Column

HiTrap Desalting 5 ml columns are made of polypropylene, which is biocompatible and non-interactive with biomolecules. The columns have porous top and bottom frits that allow high flow rates. It is delivered with a stopper on the inlet and a snap off end on the outlet.

The separation can be easily achieved using a syringe together with the supplied luer adaptor, a peristaltic pump, or in a chromatography system as ÄKTA design.

Note: To prevent leakage it is essential to ensure that the adaptor is tight.

The column cannot be opened or refilled.

Table 1. HiTrap Desalting characteristics

Matrix	Sephadex G-25 Superfine, cross-linked dextran
Separation mechanism	According to size
Column volume	5 ml
Column 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 × applied volume
Exclusion limit	M_r 5 000
Bead size	15–70 µm
Maximum flow rate*	15 ml/min
Recommended flow rate*	1–10 ml/min
Back pressure at 10 ml/min*	0.25 bar
Maximum backpressure	3 bar, 0.3 MPa
Chemical stability	All commonly used buffers
pH stability, short and long term	2–13
Storage	20% ethanol

* room temperature, aqueous buffers

** *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 subsequent chromatographic performance.

2. Operation

Buffer preparation

For substances carrying charged groups an eluent containing a buffer salt is recommended. A salt concentration of at least 25 mM is recommended to prevent possible ionic interactions with the matrix. Sodium chloride is often used for this purpose. At salt concentrations above 1.0 M, hydrophobic substances may be retarded or bind to the matrix. At even higher salt concentrations (>1.5 M $(NH_4)_2SO_4$), the column packing shrinks.

Sample preparation

The sample concentration does not influence the separation as long as the viscosity does not differ more than a factor of 1.5 from that of the buffer used. This corresponds to a maximum concentration of 70 mg/ml for proteins or 5 mg/ml for high molecular weight polymers such as dextran, when normal aqueous buffers are used. The sample should be fully solubilized. Centrifuge or filter (0.45 µm filter) immediately before loading to remove particulate material if necessary.

Method

Column equilibration

1. Fill the syringe or pump tubing with buffer. Remove the stopper. To avoid introducing air into the column, connect the column “drop to drop” to either the syringe (via the adaptor) or to the pump tubing.
2. Remove the snap-off end at the column outlet.
3. Equilibrate the column with 25 ml buffer at 5 ml/min to completely remove the ethanol.

Note: 5 ml/min corresponds to approx. 120 drops/min when using a HiTrap 5 ml column.

If air is trapped in the column, wash with degassed buffer until the air disappears. Inverting the column while washing enables the air to escape more easily through the column outlet. Air introduced onto the column by accident during sample application do not influence the separation.

Operation with a syringe

To operate the column with a syringe, connect the syringe to the column with the supplied luer adaptor.

1. Equilibrate the column; see Column equilibration.
2. Apply the sample using a 2–5 ml syringe.

   The maximum recommended sample volume is 1.5 ml. See Figure 1 for the effect of varying the sample volume applied to the column using a syringe. Use a flow rate between 1–10 ml/min. Discard the eluted buffer from the column.
3. Change to buffer and proceed with injection.

   If the sample volume is less than 1.5 ml, add buffer until a total of 1.5 ml buffer is eluted. Discard the eluted buffer.
4. Elute the high molecular weight components with the volumes listed in Table 2. *Collect the eluted buffer.*

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

Sample load ml	Elute and Add buffer ml	collect ml	Yield %	Remaining 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	0.0	2.0	>95	<0.2	1.3

Note: Certain types of molecules, such as small heterocyclic or homocyclic aromatic compounds (purines, pyrimidines, dye stuffs) can interact with Sephadex and are therefore eluted later than expected. Larger sample volumes can be used in these cases, but the separation has to be optimized for each case.

Operation with a pump or a chromatography system

The void volume of the column is 1.5 ml. High molecular weight components elute between 1.5 and 4.5 ml, depending on the sample volume.

Low molecular weight components start to elute after 3.5 ml. See Figure 2 for the effect of varying the sample volume applied to the column using tubing sample loops.

1. Equilibrate the column; see Column equilibration.
2. Apply up to 1.5 ml of sample. Monitor the effluent from the column with a UV monitor and/or a conductivity monitor. Keep the flow rate in the range 1–10 ml/min. Collect fractions.
3. Elute the column with approximately 10 ml buffer before applying the next sample. Collect fractions.

Note: The method of sample injection is important for the separation. The use of tubing sample loops results in band broadening and poorer resolution in comparison to syringe sample application. This effect is illustrated in Figure 1.

3. Scaling up

For separation of sample volumes larger than 1.5 ml, or to increase the resolution between high and low molecular weight components, up to five HiTrap Desalting columns can easily be connected in series. For syringe operations, the volumes suggested in Table 2 should be increased proportionally and the recommended flow rate maintained. The dilution of the sample is dependent on the sample volume and the number of columns used in series. Lower dilution factors then those proposed in Table 2 can be obtained, but the elution volumes have to be optimized for each combination of sample volume and number of columns in series. The back pressure for each column is approximately 0.25 bar at 10 ml/min. For sample volumes up to 15 ml HiPrep™ 26/10 Desalting is available. Up to four HiPrep 26/10 Desalting columns can be connected in series without increased backpressure (up to 60 ml sample volume).

4. Storage

Store the HiTrap Desalting column equilibrated with 25 ml 20% ethanol. The recommended storage temperature is 4 to 30 °C.

5. Further information

Visit www.gelifesciences.com/hitrap for further information. Useful handbooks and guides are also available, see ordering information.

6. Ordering Information

Product	No. Supplied	Code No.
HiTrap Desalting	5 × 5 ml	17-1408-01
HiTrap Desalting	100 × 5 ml*	11-0003-29

Related products	No. Supplied	Code No.
PD-10 Desalting Column	30	17-0851-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
HiPrep 26/10 Desalting	4 × 53 ml	17-5087-02

* Special pack delivered on specific customer order.

Accessories	No. Supplied	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 stop plugs female included in HiTrap packages depending on the product.

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

Related literature

Gel Filtration Handbook, Methods and Principles	1	18-1022-18
Gel Filtration Column and Media, Selection Guide	1	18-1124-19
Convenient protein purification, HiTrap column guide	1	18-1129-81

Column:	HiTrap Desalting
Sample:	2 mg/ml bovine serum albumin in 50 mM sodium phosphate buffer, 0.5 M sodium chloride, pH 7.0
Sample volume:	0.3 ml, 1.3 ml, 2.2 ml
Eluent:	50 mM sodium phosphate buffer, 0.15 M sodium chloride, pH 7.0
Flow rate:	5 ml/min
Detection:	UV (280 nm, 5 mm cell) and conductivity
Sample injection:	Syringe

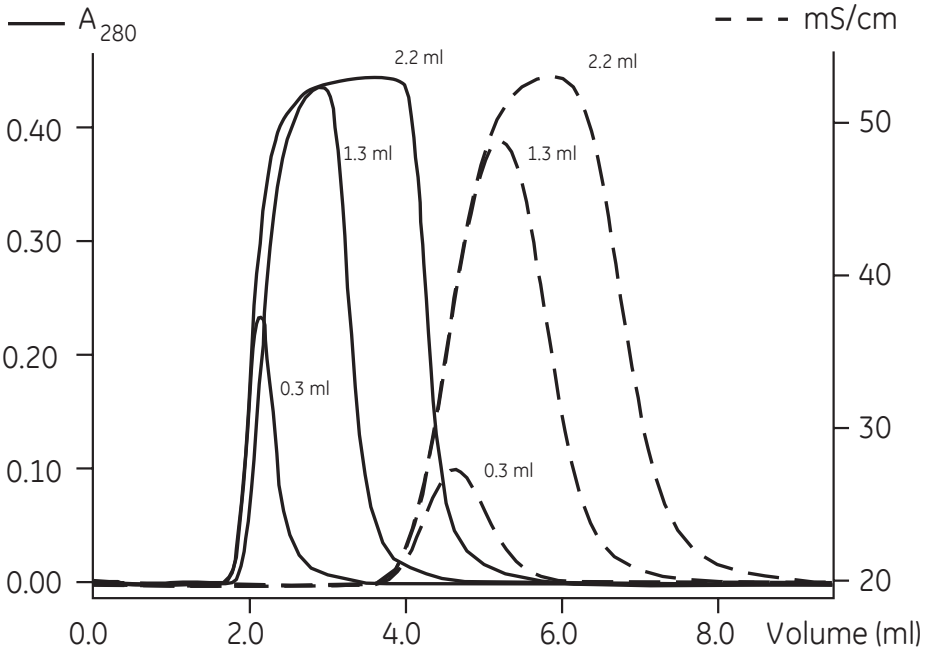

Fig 1. The effect of different sample volumes on the HiTrap Desalting column using a syringe for sample injection.

Column:	HiTrap Desalting
Sample:	2 mg/ml bovine serum albumin in 50 mM sodium phosphate buffer, 0.5 M sodium chloride, pH 7.0
Sample volume:	0.5 ml, 1.1 ml, 2.1 ml
Eluent:	50 mM sodium phosphate buffer, 0.15 M sodium chloride, pH 7.0
Flow rate:	5 ml/min
Detection:	UV (280 nm, 5 mm cell) and conductivity
Sample injection:	Tubing loops

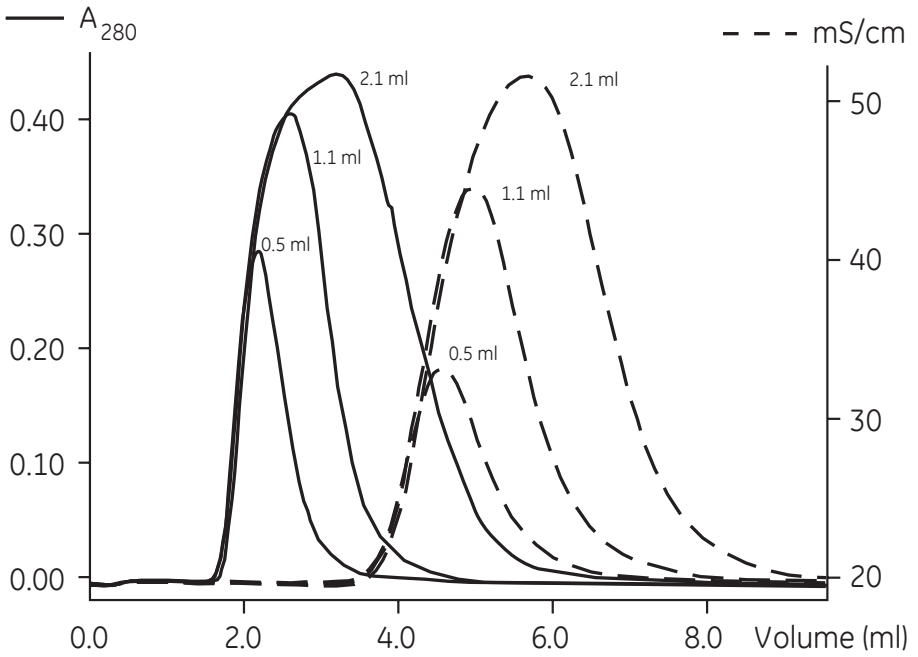

Fig 2. The effect of different sample volumes on the HiTrap Desalting column using tubing loops for sample injection.

www.gelifesciences.com/hitrap

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