cytiva

HiTrap Desalting, 5 ml

Instructions for Use

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 ÄKTA™.

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Read these instructions carefully before using HiTrap columns.

Intended use

HiTrap columns are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the product in a safe way, refer to the Safety Data Sheet.

1 Product description

HiTrap column characteristics

The columns are made of biocompatible polypropylene that does not interact with biomolecules.

The columns are delivered with a stopper at the inlet and a snap-off end at the outlet. Table 1 lists the characteristics of HiTrap columns.

Fig 1. HiTrap, 5 ml column.

Note: *HiTrap columns cannot be opened or refilled.*

Note: *Make sure that the connector is tight to prevent leakage.*

Table 1. Characteristics of HiTrap columns.

Column volume (CV)	5 ml
Column dimensions	1.6 x 2.5 cm
Column hardware pressure limit	5 bar (0.5 MPa)

Note: *The pressure over the packed bed varies depending on a range of parameters such as the characteristics of the chromatography medium, sample/liquid viscosity and the column tubing used.*

Supplied Connector kit with HiTrap column

Connectors supplied	Usage	No. supplied
Union 1/16" male/ luer female	For connection of syringe to HiTrap column	1
Stop plug female, 1/ 16"	For sealing bottom of HiTrap column	2, 5 or 7

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 and 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 to 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 to1.5 ml when complete removal of low molecular weight components is desired. The separation is not affected by the flow rate, in the range 1 to 10 ml/min. The maximum recommended flow rate is 15 ml/min. Characteristics of the HiTrap Desalting column are summarized in Table 2.

Table 2. HiTrap Desalting characteristics

Matrix	Sephadex G-25 Superfine, cross-linked dextran
Separation mechanism	According to size
Void volume	1.5 ml
Recommended sample volume	0.1 to 1.5 ml
Sample dilution, syringe operation	1.3 to 4.0 × applied volume
Exclusion limit	M_r 5 000
Bead size	15 to 70 µm
Maximum flow rate[1]	15 ml/min
Recommended flow rate[1]	1 to 10 ml/min
Back pressure at 10 ml/min[1]	0.25 bar
Chemical stability	All commonly used buffers
pH stability, short and long term[2]	2 to 13
Storage	20% ethanol at 4°C to 30°C

[1] room temperature, aqueous buffers

[2] *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 connector) 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 connector.

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 2 for the effect of varying the sample volume applied to the column using a syringe. Use a flow rate between 1 and10 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 3. **Collect the eluted buffer.**

Table 3. 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)	Remaining Yield (%)	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 3 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 to 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 3*

Examples

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

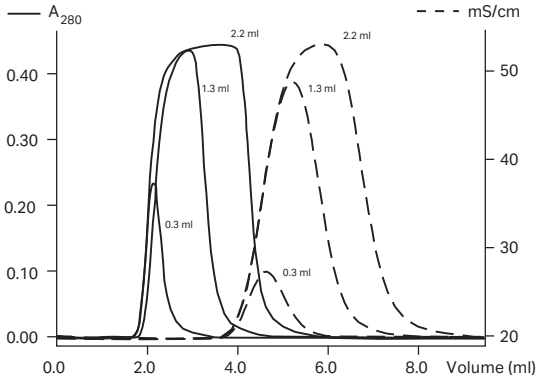

Fig 2. The effect of different sample volumes on the HiTrap Desalting column using a syringe for sample injection

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

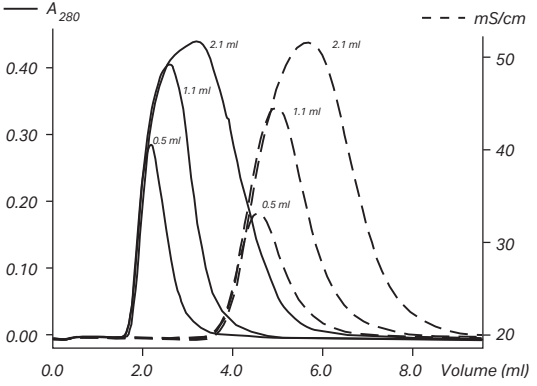

Fig 3. The effect of different sample volumes on the HiTrap Desalting column using tubing loops for sample injection.

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 3 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 3 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 Adjusting pressure limits in chromatography system software

Pressure generated by the flow through a column affects the packed bed and the column hardware, see Fig 4. Increased pressure is generated when running/using one or a combination of the following conditions:

- High flow rates
- Buffers or sample with high viscosity
- Low temperature
- A flow restrictor

Note: *Exceeding the flow limit (see Table 2) may damage the column.*

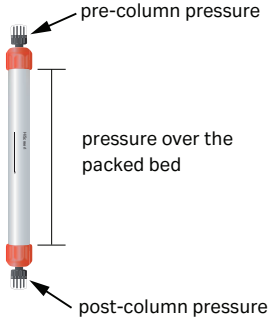

Fig 4. Pre-column and post-column measurements.

ÄKTA avant

The system will automatically monitor the pressures (pre-column pressure and pressure over the packed bed, Δp). The pre-column pressure limit is the column hardware pressure limit (see Table 1).

The maximum pressure the packed bed can withstand depends on media characteristics and sample/liquid viscosity. The measured value also depends on the tubing used to connect the column to the instrument.

ÄKTAexplorer, ÄKTApurifier, ÄKTAFPLC and other systems with pressure sensor in the pump

To obtain optimal functionality, the pressure limit in the software may be adjusted according to the following procedure:

1. Replace the column with a piece of tubing. Run the pump at the maximum intended flow rate. Note the pressure as *total system pressure*, P1.
2. Disconnect the tubing and run the pump at the same flow rate used in step 1. Note that there will be a drip from the column valve. Note this pressure as P2.
3. Calculate the new pressure limit as a sum of P2 and the column hardware pressure limit (see Table 1). Replace the pressure limit in the software with the calculated value.

   The actual pressure over the packed bed (Δp) will during run be equal to actual measured pressure - *total system pressure* (P1).

Note: *Repeat the procedure each time the parameters are changed.*

5 Storage

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

6 Ordering information

Product	No. Supplied	Code No.
HiTrap Desalting	1 × 5 ml	29-0486-84
	5 × 5 ml	17-1408-01
	100 × 5 ml[1]	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

[1] Special pack delivered on specific customer order.

Accessories	Quantity	Code No.
1/16" male/luer female *(For connection of syringe to top of HiTrap column*)	2	18-1112-51
Tubing connector flangeless/M6 female (*For connection of tubing to bottom of HiTrap column)*	2	18-1003-68
Tubing connector flangeless/M6 male *(For connection of tubing to top of HiTrap column)*	2	18-1017-98
Union 1/16" female/M6 male *(For connection to original FPLC System through bottom of HiTrap column)*	6	18-1112-57
Union M6 female /1/16" male *(For connection to original FPLC System through top of HiTrap column*)	5	18-3858-01
Union luerlock female/M6 female	2	18-1027-12
HiTrap/HiPrep, 1/16" male connector for ÄKTA design	8	28-4010-81
Stop plug female, 1/16" *(For sealing bottom of HiTrap column)*	5	11-0004-64
Fingertight stop plug, 1/16"	5	11-0003-55

Related literature	Code No.
Gel Filtration Handbook, Methods and Principles	18-1022-18
Gel Filtration Column and Media, Selection Guide	18-1124-19

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71715400 AN 06/2020