GE Healthcare

Instructions 28-4026-53 AE

HiPrep 16/60 & 26/60 Sephacryl S-100 High Resolution

HiPrep 16/60 & 26/60 Sephacryl S-200 High Resolution

HiPrep 16/60 & 26/60 Sephacryl S-300 High Resolution

HiPrep 16/60 & 26/60 Sephacryl S-400 High Resolution

HiPrep 16/60 & 26/60 Sephacryl S-500 High Resolution

Introduction

HiPrep™ 16/60 and 26/60 Sephacryl™ S-100 High Resolution (HR), Sephacryl S-200 HR, Sephacryl S-300 HR, Sephacryl S-400 HR and Sephacryl S-500 HR are prepacked gel filtration columns designed for preparative purification of peptides, proteins and larger molecules.

Sephacryl High Resolution is a cross-linked copolymer of allyl dextran and N,N-methylenebisacrylamide. This cross-linking gives the matrix good ridgity and chemical stability.

Steep selectivity curves give excellent resolution power for peptides and proteins in the molecular weight range, M_r 1 000–100 000 (Sephacryl S-100), M_r 5 000–250 000 (Sephacryl S-200), M_r 10 000–1 500 000 (Sephacryl S-300), M_r 20 000–8 000 000 (Sephacryl S-400) and dextrans in the range M_r 40 000–20 000 000 (Sephacryl S-500).

See Figures 1 and 2, and "Column data" for column characteristics.

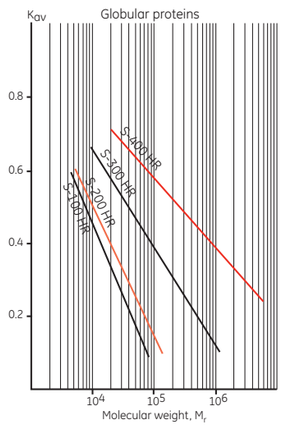


Fig 1. Selectivity curves for globular proteins in 0.05 M phosphate buffer, 0.15 M NaCl, pH 7.0 on Sephacryl HR media.

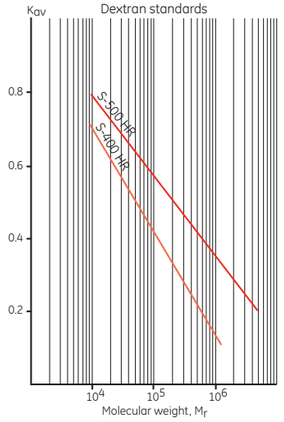


Fig 2. Selectivity curves for dextran standards in 0.05 M phosphate buffer, 0.15 M NaCl, pH 7.0 on Sephacryl HR media.

Column data

Matrix	Cross-linked copolymer of allyl dextran and N,N-methylenebisacrylamide
Mean particle size	47 µm (25–75 µm)
Separation range (M_r)	
globular proteins	1×10^3–1×10^5 (Sephacryl S-100 HR) 5×10^3–2.5×10^5 (Sephacryl S-200 HR) 1×10^4–1.5×10^6 (Sephacryl S-300 HR) 2×10^4–8×10^6 (Sephacryl S-400 HR)
dextrans	1×10^3–8×10^4 (Sephacryl S-200 HR) 2×10^3–4×10^5 (Sephacryl S-300 HR) 1×10^4–2×10^6 (Sephacryl S-400 HR) 4×10^4–2×10^7 (Sephacryl S-500 HR)
Column volume	120 ml (16/60) 320 ml (26/60)
Sample volume[1]	Up to 5 ml (HiPrep 16/60) Up to 13 ml (HiPrep 26/60)
Recommended flow rate	15 cm/h at room temperature (0.5 ml/min for 16/60 or 1.3 ml/min for 26/60)
Maximum flow rate	30 cm/h at room temperature (1 ml/min for 16/60 or 2.6 ml/min for 26/60)
Maximum pressure over the packed bed during operation[2]	0.15 MPa, 1.5 bar, 21 psi
HiPrep column hardware pressure limit[2]	0.5 MPa, 5 bar, 73 psi
Theoretical plates	> 5 000 m^{-1}
pH stability	
long term and working range	3–11
short term	2–13
Storage	4°C to 30°C in 20% ethanol

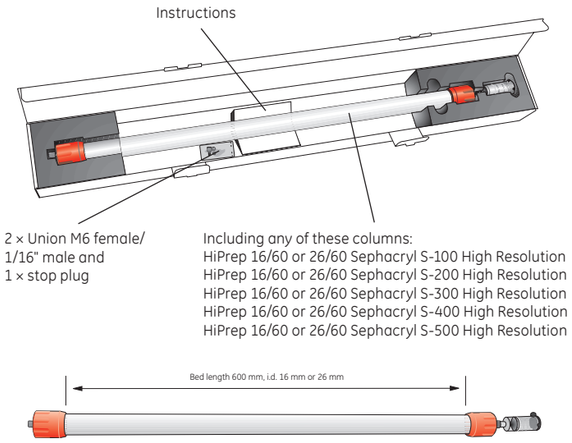
[1] Optimal sample volume depends on the complexity of the sample, and flow rate. If the sample contains substances with small differences in size, either decrease sample volume, or decrease flow rate (in very difficult cases, it may be necessary to decrease both).

[2] Many chromatography systems are equipped with pressure gauges to measure pressure at a particular point in the system, usually just after the pumps. The pressure measured here is the sum of pre-column pressure, pressure drop over the medium bed, and post-column pressure. This is always higher than the pressure drop over the bed alone. Keeping the pressure drop over the bed below 1.5 bar is recommended. Setting the upper limit of the pressure gauge to 1.5 bar will ensure that the pump shuts down before the medium is overpressured. If necessary, post-column pressure of up to 3.5 bar can be added to the limit without exceeding the column hardware limit.
To avoid breaking the column, post-column pressure must never exceed 3.5 bar.

To determine post-column pressure, proceed as follows:

1. Connect a piece of tubing in place of the column.
2. Run the pump at the maximum flow you intend to use for chromatography. Use a buffer with the same viscosity as you intend to use for chromatography. Note the backpressure as total pressure.
3. Disconnect the tubing and run at the same flow rate used in step 2. Note this backpressure as pre-column pressure.
4. Calculate post-column pressure as total pressure minus pre-column pressure.

If post-column pressure is higher than 3.5 bar, take steps to reduce it (shorten tubing, clear clogged tubing, or change flow restrictors), and perform steps 1–4 again until the post-column pressure is below 3.5 bar. Note post-column pressure when it has reached a satisfactory level, add 1.5 bar to this value, and set this as the upper pressure limit on the chromatography system.



First time use

These HiPrep columns can be used directly on ÄKTA™ design systems without the need for any extra connectors.

Connecting the column

1. Before connecting the column to a chromatography system, start the pump to remove all air from the system, particularly in tubing and valves.
2. Stop the pump.
3. Mount the column vertically, remove the bottom stop plug, and connect the inlet tubing to the system "drop-to-drop".
4. Remove the transport device and connect the column outlet tubing to, for example, a monitor cell. Save the transport device for use when storing the column. The column is now ready for use.

Equilibration of the column

Ensure an appropriate pressure limit has been set. Equilibrate the column for first-time use, or after long-term storage as follows:

1. One-half column volume of distilled water at a flow rate of 15 cm/h (0.5 ml/min for 16/60 or 1.3 ml/min for 26/60).
2. Two column volumes of buffer, e.g. 0.05 M sodium phosphate, 0.15 M NaCl, pH 7.2 at 30 cm/h (1 ml/min for 16/60 or 2.6 ml/min for 26/60).

Note: Recommended flow rates are valid for H_2O at 25° C.

Try these conditions first

Flow rate	15 cm/h (0.5 ml/min for 16/60, or 1.3 ml/min for 26/60).
Sample volume	1% of the column volume (1.2 ml for 16/60 or 3.2 ml for 26/60)
Buffer	0.05 M sodium phosphate, 0.15 M NaCl, pH 7.2 or select the buffer in which the sample should be solubilized for the next step. To avoid pH-dependent non-ionic interactions with the matrix, include at least 0.15 M salt in the buffer (or use buffer with equivalent ionic strength).

Note: Recommended flow rates are valid for H_2O at 25° C.

Read the section "Optimization" for information on how to optimize a separation.

Regeneration before a new run

Regenerate the column after each run with one column volume of start buffer at 30 cm/h (1 ml/min for 16/60 or 2.6 ml/min for 26/60).

Buffers and solvent resistance

De-gas and filter all solutions through 0.22 µm filter to increase column lifetime. Buffers and solvents with increased viscosity will affect the backpressure and flow rate.

Daily use

All commonly used aqueous buffers, pH 3–11

Cleaning

Acetonitrile, up to 30%
Sodium hydroxide, up to 0.5 M
Ethanol, up to 24%
Acetic acid, up to 1 M
Isopropanol, up to 30%
Guanidine hydrochloride, up to 6 M
Urea, up to 8 M

Avoid

Unfiltered solutions

Sample recommendations

Recommended sample load	0.5%–4% of the column volume (0.6 –4.8 ml for 16/60, or 1.6–12.8 ml for 26/60) **Note:** The sample volume is critical for the resolution during the purification.
Preparation	Dissolve the sample in start buffer, filter through 0.22 µm filter, or centrifuge at 10 000 x g for 10 min.

Delivery and storage

The prepacked column is supplied in 20% ethanol. If the column is to be stored more than 2 days after use, wash the column with four column volumes of distilled water, followed by four column volumes of 20% ethanol.

To avoid air bubble formation in the column, use the transport device. Connect the transport device to the capillary tubing at the column outlet. Start the pump, and fill to approximately 50% of the total device volume.

Note: HiPrep columns cannot be opened or refilled.

Choice of buffer

Selection of buffering ion does not directly affect resolution. Select a buffer in which the purified product should be collected, and which is compatible with protein stability and activity. Buffer concentration must be sufficient to maintain buffering capacity and constant pH. Ionic strength should be at least 0.15 M NaCl in the buffer, to avoid non-specific ionic interactions with the matrix.

Optimization

Perform a first run as described in the section "Try these conditions first". If the obtained results are unsatisfactory, consider the following:

Action	Effect
Decrease flow rate	Improved resolution
Decrease sample volume	Improved resolution

HiPrep

Cleaning-in-place (CIP)

Regular cleaning

Wash the column with one-half column volume of 0.2 M NaOH at a flow rate of 15 cm/h (0.5 ml/min for 16/60 or 1.3 ml/min for 26/60) to remove most proteins non-specifically bound to the medium. After cleaning, immediately equilibrate the column with at least two column volumes of buffer. Further equilibration is necessary if your buffer contains detergent. Wait until the UV baseline has stabilized before applying next sample.

More rigorous cleaning

Wash the column at a flow rate of 10 cm/h (0.3 ml/min for 16/60 or 0.8 ml/min for 26/60) at room temperature with the following solutions:

1. One-quarter of a column volume 0.5 M NaOH (removal of hydrophobic proteins or lipoproteins), followed by four column volumes of distilled water.
2. One-half column volume 30% isopropanol (removal of lipids and very hydrophobic proteins), followed by four column volumes distilled water.
3. To remove precipitated proteins, digest the protein with one column volume pepsin (1 mg/ml in 0.1 M acetic acid, 0.5 M NaCl) overnight at room temperature. Wash with one-half column volume 0.2 M NaOH at 15 cm/h (0.5 ml/min for 16/60 or 1.3 ml/min for 26/60) to remove trace amounts of enzyme remaining in the system, followed by four column volumes distilled water.

If a new purification is to be run, equilibrate the column after cleaning with at least five column volumes of buffer.

Note: HiPrep columns cannot be opened or refilled.

Troubleshooting

Symptom	Remedy
Increased backpressure over the column	Clean the column according to the section "Cleaning-in-place (CIP)".
Loss of resolution and/or decreased sample recovery	Clean the column according to the section "Cleaning-in-place (CIP)".
Air in the column	Reverse the flow direction, and pump five column volumes of well de-gassed water through the column at a flow rate of 30 cm/h (1 ml/min for 16/60 or 2.6 ml/min for 26/60).

Column efficiency test

We recommend checking the column performance at regular intervals. Figure 3 describes how to check the performance of HiPrep 16/60 and HiPrep 26/60 Sephacryl columns.

Column efficiency is calculated using the equation:

$$H^{-1} = 5.54\,(V_R/w_h)^2\ 1000/L$$

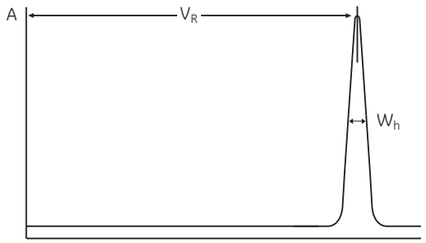
where,

V_R = peak retention (elution) volume

w_h = peak width at half peak height

L = bed height (mm)

V_R and w_h in same units



Example conditions:

Sample	2% acetone in 20% ethanol
Sample volume	200 µl (XK 16/60) and 500 µl (XK 26/60)
Eluent	20% ethanol
Flow rate	15 cm/h 0.5 ml/min (16/60) and 1.3 ml/min (26/60)
Detection	280 nm (5 mm cell, 0.2 AUFS)
Temperature	Room temperature (25°)

Fig 3. Column efficiency test .

Examples of column performance

Below are examples of performance for the different HiPrep Sephacryl columns, using standard proteins or dextrans, see Figures 4 a-e.

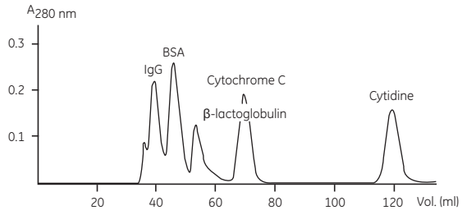
Conditions for Figures 4 a-c

Columns	a) HiPrep 16/60 Sephacryl S-100 HR b) HiPrep 16/60 Sephacryl S-200 HR c) HiPrep 16/60 Sephacryl S-300 HR
Sample	500 µl of a mixture containing IgG (M_r 160 000), BSA (M_r 67 000), β-lactoglobulin (M_r 35 000), cytochrome C (M_r 12 400) and cytidine (M_r 240)
Buffer	0.05 M sodium phosphate, 0.15 M NaCl, pH 7.0
Flow rate	0.8 ml/min (24 cm/h)

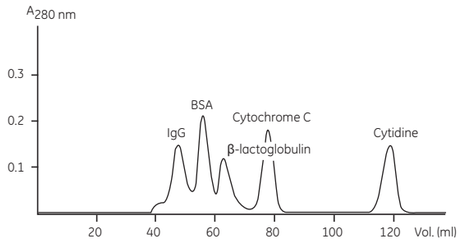
Conditions for Figures 4 d-e

Columns	d) HiPrep 16/60 Sephacryl S-400 HR e) HiPrep 16/60 Sephacryl S-500 HR
Sample	1.2 ml of a mixture containing three dextrans: d) M_r > 20×10^6, M_r 270 000 and M_r 12 000 e) M_r > 20×10^6, M_r 1 800 000 and M_r 25 000
Buffer	0.25 M NaCl
Flow rate	0.5 ml/min (15 cm/h)

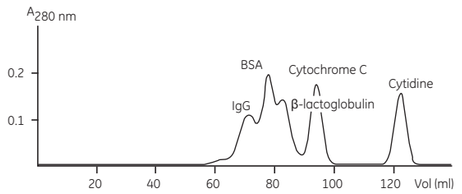
a) HiPrep 16/60 Sephacryl S-100 HR



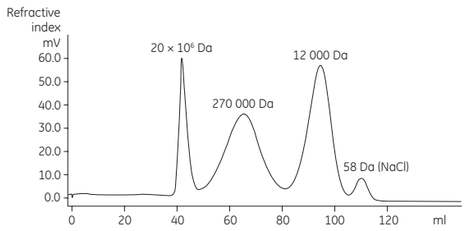
b) HiPrep 16/60 Sephacryl S-200 HR



c) HiPrep 16/60 Sephacryl S-300 HR



d) HiPrep 16/60 Sephacryl S-400 HR



e) HiPrep 16/60 Sephacryl S-500 HR

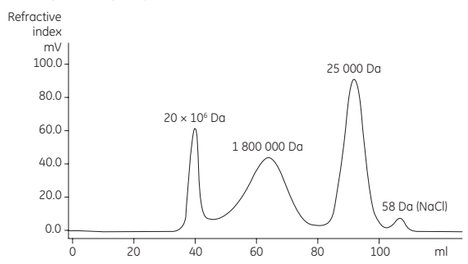


Fig 4 a-e. Examples of column performance for different HiPrep Sephacryl columns.

Intended use

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

Ordering information

Products	No. supplied	Code No.
HiPrep 16/60 Sephacryl S-100 High Resolution	1 × 120 ml	17-1165-01
HiPrep 26/60 Sephacryl S-100 High Resolution	1 × 320 ml	17-1194-01
HiPrep 16/60 Sephacryl S-200 High Resolution	1 × 120 ml	17-1166-01
HiPrep 26/60 Sephacryl S-200 High Resolution	1 × 320 ml	17-1195-01
HiPrep 16/60 Sephacryl S-300 High Resolution	1 × 120 ml	17-1167-01
HiPrep 26/60 Sephacryl S-300 High Resolution	1 × 320 ml	17-1196-01
HiPrep 16/60 Sephacryl S-400 High Resolution	1 × 120 ml	28-9356-04
HiPrep 26/60 Sephacryl S-400 High Resolution	1 × 320 ml	28-9356-05
HiPrep 16/60 Sephacryl S-500 High Resolution	1 × 120 ml	28-9356-06
HiPrep 26/60 Sephacryl S-500 High Resolution	1 × 320 ml	28-9356-07

Accessories	No. supplied	Code No.
HiTrap™/HiPrep 1/16" male connector to ÄKTA design	8	28-4010-81
Union M6 female/1/16" male* (for connection to FPLC™ Systems)	5	18-3858-01

* 2 unions (in red polypropylene) are included in HiPrep package.

Related literature	Code No.
Gel Filtration Handbook, Principles and Methods	18-1022-18
Gel Filtration Columns and Media, Selection Guide	18-1124-19
Prepacked chromatography columns for ÄKTA design systems, Selection Guide	28-9317-78

Further information

For more information refer to related literature (see above) or visit:
www.gelifesciences.com/protein-purification

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

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