

HiLoad 16/10 and 26/10 Q Sepharose High Performance

HiLoad 16/10 and 26/10 SP Sepharose High Performance

Introduction

HiLoad™ 16/10 and 26/10 Q Sepharose™ High Performance, and HiLoad 16/10 and 26/10 SP Sepharose High Performance are prepacked XK columns designed for preparative ion exchange chromatography separations. Q and SP Sepharose HP are strong ion exchangers based on highly cross-linked agarose beads. The functional group on the matrix for Q Sepharose HP is a quaternary amino group, while the functional group on the matrix for SP Sepharose HP is a sulphonate group. The functional groups are coupled to the matrices via chemically stable ether linkages. High mechanical strength of the matrix allows for very high flow rates, with superior resolution.

See "Column data" below for column characteristics.

Column data

Matrix	6% highly cross-linked spherical agarose	
Mean particle size	34 µm (24–44 µm)	
Column volume ¹	20–22 ml (XK 16/10) 53–58 ml (XK 26/10)	
Theoretical plates	>12 000 m ⁻¹	
Recommended flow rate	Up to 150 cm/h at room temperature	
Maximum flow rate	150 cm/h (5 ml/min for XK 16/10, or 13 ml/min for XK 26/10)	
Maximum pressure over the packed bed during operation ³	0.3 MPa, 3 bar, 42 psi	
HiLoad column hardware pressure limit ²	0.5 MPa, 5 bar, 73 psi	
Type of ion exchanger	Q Strong anion	SP Strong cation
Charged group	-CH ₂ N ⁺ (CH ₃) ₃	-CH ₂ CH ₂ CH ₂ SO ₃ ⁻
Total ionic capacity (mmol/ml medium)	0.14–0.20 (Cl ⁻)	0.15–0.20 (H ⁺)
Dynamic binding capacity ²	60 mg BSA/ml medium	55 mg Ribonuclease A/ml medium
pH stability		
long term and working range	2–12	4–13
short term	1–14	3–14
Storage	20% ethanol	20% ethanol, 0.2 M sodium acetate

¹ The medium surface is not entirely visible at the bottom piece. Therefore, when calculating the total column volume, measure the height from the lowest part of the bottom piece to the medium/adaptor surface. For HiLoad 16/10 deduct 30 mm, and for HiLoad 26/10 deduct 36 mm.

² Dynamic binding capacity was determined at 150 cm/h using 10 mg/ml BSA in 20 mM Tris-HCl, pH 8.2, and 5.0 mg/ml ribonuclease A in 100 mM sodium acetate, pH 6.0.

³ In exceptional circumstances, the packed bed can withstand pressures of up to 0.4 MPa, 4 bar, 56 psi for short periods.

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 3 bar is recommended. Setting the upper limit of the pressure gauge to 3 bar will ensure that the pump shuts down before the medium is overpressured. If necessary, post-column pressure of up to 2 bar can be added to the limit without exceeding the column hardware limit. To determine post-column pressure, proceed as follows:

To avoid breaking the column, post-column pressure must never exceed 2 bar.

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 2 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 2 bar. Note post-column pressure when it has reached a satisfactory level, add 3 bar to this value, and set this as the upper pressure limit on the chromatography system.

First-time use

Connecting the column

1. Before connecting the column to a chromatography system, start the pump to remove all air or from the system, particularly in tubing and valves.
2. Stop the pump.
3. Mount the column vertically, remove the domed nut, and connect the inlet tubing to the system "drop-to-drop".
4. Remove the transport syringe and connect the column outlet tubing to, for example, a monitor cell. Save the transport syringe 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 column volume of distilled water to wash out the ethanol at 25 cm/h (0.8 ml/min for XK 16/10 or 2.2 ml/min for XK 26/10).
2. Five column volumes of start buffer at 50 cm/h (1.6 ml/min for XK 16/10 or 4.3 ml/min for XK 26/10), see section "Choice of buffer" for buffer recommendations.
3. Five column volumes of elution buffer at 100 cm/h (3.2 ml/min for XK 16/10 or 8.5 ml/min for XK 26/10).
4. Five column volumes of start buffer at 100 cm/h (3.2 ml/min for XK 16/10 or 8.5 ml/min for XK 26/10).

Try these conditions first

Flow rate: 50–100 cm/h (1.6–3.2 ml/min for XK 16/10 or 4.3–8.5 ml/min for XK 26/10).

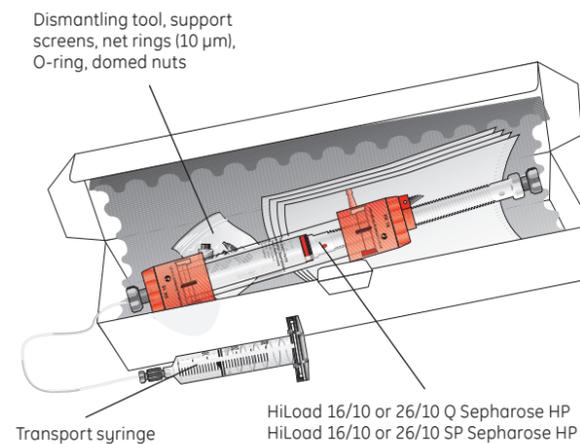
Gradient volume: 10–20 column volumes

Start buffer: See section "Choice of buffer"

Elution buffer: Start buffer + 1 M NaCl

Equilibration before a new run

Proceed according to steps 3 and 4 in the section "Equilibration of the column". Extended equilibration may be needed if detergents were included in the eluent. Please read the section "Optimization" for information on how to optimize a separation.



Buffers and solvent resistance

De-gas and filter all solutions through 0.22 µm filter to increase column lifetime.



Daily use

All commonly used aqueous buffers (see "Column data" for recommended pH)
Urea, up to 8 M
Guanidine hydrochloride, up to 6 M

Cleaning

Sodium hydroxide, up to 1 M
Ethanol, up to 70%
Acetic acid, up to 1 M
Isopropanol, up to 30%
Acetonitrile, up to 30%
SDS, up to 2%

Avoid

Oxidizing agents
Cationic detergents and buffers (SP)
Anionic detergents and buffers (Q)
Unfiltered solutions

Sample recommendations

Net charge of protein: Positive (SP), negative (Q)
Recommended sample load: Up to 600 mg for XK 16/10, Up to 1600 mg for XK 26/10

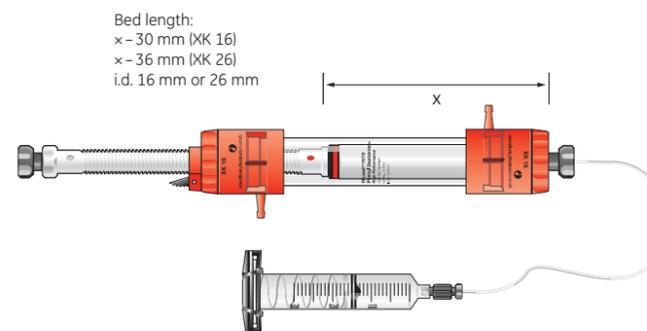
Preparation

Note: Sample volume is **not** critical for the separation.
Dissolve the sample in start buffer, filter through 0.22 µm filter, or centrifuge at 10 000 × g for 10 min.

Delivery and storage

HiLoad 16/10 and 26/10 Q Sepharose HP are supplied in 20% ethanol. HiLoad 16/10 and 26/10 SP Sepharose HP are supplied in 20% ethanol, 0.2 M sodium acetate. If the column is to be stored more than two days after use, clean the column according to the procedure described under "Cleaning-in-place" (CIP). Then equilibrate with at least five column volumes of 20% ethanol (Q) or 20% ethanol, 0.2 M sodium acetate (SP) at 50–100 cm/h (1.6–3.2 ml/min for XK 16/10, or 4.3–8.5 ml/min for XK 26/10) at room temperature.

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



Choice of buffer

To avoid local disturbances in pH caused by buffering ions participating in the ion exchange process, select an eluent with buffering ions of the same charge as the substituent groups on the ion exchanger.

The start buffer pH should be chosen so that substances to be bound to the ion exchanger are charged, that is at least 1 pH unit above the isoelectric point for anion exchangers or at least 1 pH unit below the isoelectric point for cation exchangers. Figure 1 and Figure 2 list a selection of standard aqueous buffers. Table 1 lists suggested volatile buffers used in cases where the purified substance has to be freeze-dried.

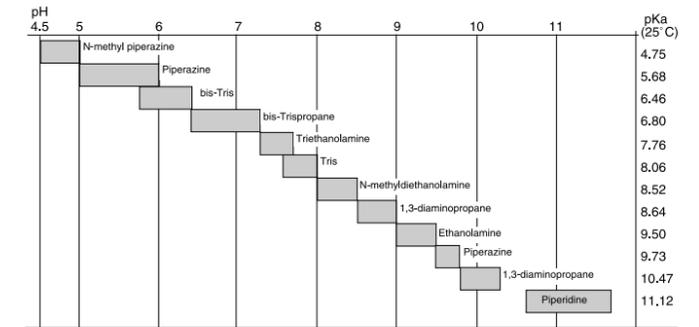


Fig 1. Recommended buffers for anion exchange chromatography.

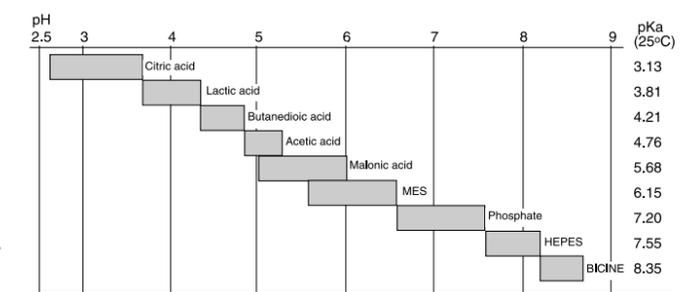


Fig 2. Recommended buffers for cation exchange chromatography.

Table 1. Volatile buffer systems.

pH	Substances
2.3–3.5	Pyridine/formic acid
3.0–5.0	Trimethylamine/formic acid
4.0–6.0	Trimethylamine/acetic acid
6.8–8.8	Trimethylamine/HCl
7.0–8.5	Ammonia/formic acid
8.5–10.0	Ammonia/acetic acid
7.0–12.0	Trimethylamine/CO ₂
8.0–9.5	Ammonium carbonate/ammonia
8.5–10.5	Ethanolamine/HCl

Optimization

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

Action	Effect
Change pH/buffer salt (see Figs. 1 and 2 for buffers)	Selectivity change, weaker/stronger binding
Change salt, counter ions and/or co-ions	Selectivity change
Smaller sample loading	Improved resolution
Lower flow rate	Improved resolution, when running isocratically
Shallower gradient	Improved resolution, but broader peaks, and decreased concentration in fractions

Cleaning-in-place (CIP)

Regular cleaning

Wash the column with two column volumes of 2 M NaCl at a flow rate of 50 cm/h (1.6 ml/min for XK 16/10, or 4.3 ml/min for XK 26/10) at room temperature. If detergents have been used, wash the column with five column volumes of distilled water followed by two column volumes of 2 M NaCl at a flow rate of 50 cm/h (1.6 ml/min for XK 16/10, or 4.3 ml/min for XK 26/10).

Re-equilibrate the column with at least five column volumes of start buffer or until the UV baseline, and pH/conductivity values have stabilized.

More rigorous cleaning

Reverse the flow direction and run at a flow rate of 25 cm/h (0.8 ml/min for XK 16/10 or 2.2 ml/min for XK 26/10) at room temperature. Use the following sequence of solutions.

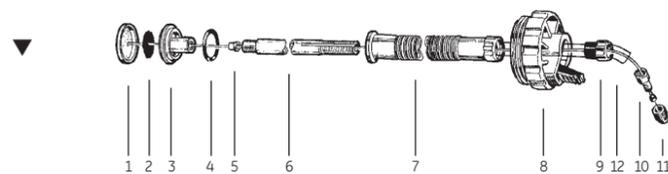
- Four column volumes of 2 M NaCl (removes ionically bound proteins) followed by two column volumes of distilled water.
- Four column volumes of 1 M NaOH solution (removes precipitated proteins, hydrophobically bound proteins, and lipoproteins) followed by four column volumes of distilled water.
- Two column volumes of 30% isopropanol (removes proteins, lipoproteins, and lipids that are strongly hydrophobically bound) followed by four column volumes of distilled water.

After cleaning, equilibrate the column before use with approximately five column volumes of start buffer at 25 cm/h (0.8 ml/min for XK 16/10 or 2.2 ml/min for XK 26/10) at room temperature in the normal flow direction.

Changing the adaptor net ring

If, after following cleaning procedures, backpressure remains too high, change the net ring in the column adaptor. Follow the instructions below thoroughly since column efficiency is easily impaired if careless measures are taken. Use distilled water as eluent.

- Close the outlet tubing of the column with a domed nut, and mark the level of the medium surface on the glass tube using a coloured pen.
- Slacken the adaptor O-ring slightly by turning the black adjusting knob counter-clockwise. Note: It should still seal against the glass wall but allow the adaptor to slide. Unscrew the top piece from the column.
- Connect the adaptor to the pump and start pumping at a flow rate of 30 cm/h (1 ml for XK 16/10, or 3 ml/min for XK 26/10). Let the flow push the adaptor upwards.
- When the glass tube is completely full, take out the adaptor and stop the pump. The glass tube should remain completely full of liquid while changing the adaptor net ring.
- To avoid getting air bubbles under the net, injection of 20% ethanol through the adaptor by a syringe is recommended.
- Insert the adaptor into the column at an angle of 45°, avoiding air bubbles. Slide the plunger 1–2 cm down and tighten the O-ring. Remove excess liquid completely before screwing the top piece onto the column end piece.
- Remove the syringe and slide the adaptor down until it touches the medium surface. Tighten the O-ring and re-connect the inlet tubing to the system, avoiding air bubbles.
- Remove the domed nut and start the pump. Increase the flow rate until the medium surface is approximately 3 mm above the pen mark. Stop the pump and close the outlet tubing with the domed nut again. Note: Step 8 requires a pump with high flow rate capacity up to a pressure of 0.5 MPa, 5 bar.
- Disconnect the inlet tubing and slacken the adaptor O-ring slightly by turning the adjusting knob counter-clockwise. Press the adaptor downwards to the pen mark. Tighten the O-ring. Note: Do not to loosen the O-ring too much as this will result in medium passing the O-ring.
- Re-connect the inlet tubing, and avoid introducing air into the system.



- | | | |
|-------------------|-------------------------|-----------------------|
| 1. Net ring | 5. Sealing plug | 9. Adjusting knob |
| 2. Support screen | 6. Inner adjusting | 10. Capillary tubing |
| 3. Plunger | 7. Outer adjusting tube | 11. Domed nut |
| 4. O-ring | 8. Top piece | 12. Protection tubing |

Troubleshooting

Symptom	Remedy
Increased back pressure 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 5 column volumes of well de-gassed start buffer through the column, at a flow rate of 150 cm/h (5 ml/min for XK 16/10 or 13 ml/min for XK 26/10).
Space between adaptor and medium	Close the outlet tubing with the domed nut and then disconnect the inlet tubing. Slacken the O-ring slightly by turning the adjusting knob counter-clockwise and push or screw the adaptor down until it touches the medium surface. Tighten the O-ring. To maintain an airtight system, reconnect the inlet tubing immediately.

Column efficiency test

GE Healthcare packs columns to the highest standard, and each column is thoroughly tested, regarding the number of theoretical plates per metre (H^{-1}), see Figure 3.

Sample	Acetone 20 mg/ml
Sample volume	200 µl (XK 16/10) and 500 µl (XK 26/10)
Eluent	Distilled water
Linear flow rate	60 cm/h

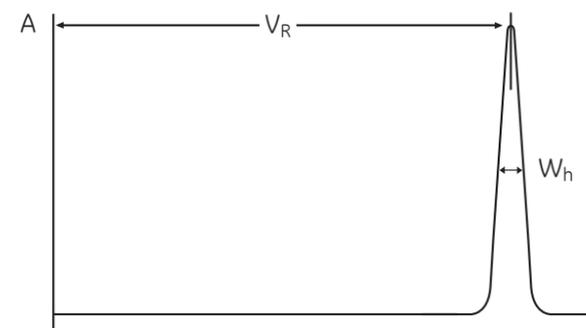


Fig 3. Column efficiency test.

The efficiency of the column 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

Ordering information

Product	No. per pack	Code No.
HiLoad 16/10 Q Sepharose High Performance	1 (20 ml)	17-1064-01
HiLoad 26/10 Q Sepharose High Performance	1 (53 ml)	17-1066-01
HiLoad 16/10 SP Sepharose High Performance	1 (20 ml)	17-1137-01
HiLoad 26/10 SP Sepharose High Performance	1 (53 ml)	17-1138-01

Companion products

Product	No. per pack	Code No.
HiTrap™ IEX Selection Kit (7 different IEX media)	7 × 1 ml	17-6002-33
HiTrap SP HP	5 × 1 ml	17-1151-01
HiTrap SP HP	5 × 5 ml	17-1152-01
HiTrap Q HP	5 × 1 ml	17-1153-01
HiTrap Q HP	5 × 5 ml	17-1154-01
HiTrap Desalting	5 × 5 ml	17-1408-01
HiPrep™ 26/10 Desalting	1 (53 ml)	17-5087-01
HiPrep 26/10 Desalting	4 (53 ml)	17-5087-02

Accessories

Product	No. supplied	Code No.
Dismantling tool*	1	-
Support screen XK 16*	2	19-0651-01
Support screen XK 26*	2	18-9377-01
Net ring (10 µm) XK 16*	2	18-8761-01
Net ring (10 µm) XK 26*	2	18-8760-01
Transport syringe*	1	18-1017-61
O-ring XK16*	1	19-0163-01
O-ring XK 26*	1	19-0688-01
Domed nut*	2	18-2450-01
Union M6 female/1/16" male (for connection to AKTA™ systems)	2	18-3858-01

* included in HiLoad 16/10 and/or 26/10 Q and SP Sepharose HP

Related printed literature

Product	No. per pack	Code No.
Handbook, Ion Exchange Chromatography & Chromatofocusing, Principles and Methods	1	11-0004-21
Ion Exchange Chromatography, Columns and Media Guide	1	18-1127-31

Further information

For more information, please visit:
www.gehealthcare.com/protein-purification to refer to the different handbooks available at GE Healthcare.

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