GE Healthcare

Instructions 28-9413-21 AC

HiPrep SP XL 16/10
HiPrep Q XL 16/10

Introduction

HiPrep™ SP XL 16/10 and HiPrep Q XL 16/10 are prepacked, ready to use columns for ion exchange chromatography. They provide fast, preparative separations of proteins and other biomolecules. See table below for column characteristics.

Column data

Matrix	6% highly cross-linked spherical agarose	
Mean particle size	90 µm	
Bed volume	20 ml	
Bed height	100 mm	
i.d.	16 mm	
Column composition	Polypropylene	
Recommended flow rate*	2–10 ml/min (30-300 cm/h)	
Maximum flow rate*	10 ml/min (300 cm/h)	
Maximum pressure over the packed bed during operation, Δp‡	0.15 MPa, 1.5 bar, 22 psi	
HiPrep column hardware pressure limit‡	0.5 MPa, 5 bar, 73 psi	
Storage	4°C to 30°C in 20% ethanol (Q) and 20% ethanol, 0.2 M sodium acetate (SP)	
	SP	**Q**
Type of exchanger	strong cation	strong anion
Charged group	$-SO_3^-$	$-N^+(CH_3)_3$
pH working range		
Short term	3–14	2–14
Working	4–13	2–12
Long term	4–13	3–13
Total ionic capacity	0.18–0.25 (mmol H^+/ml medium)	0.18–0.26 (mmol Cl^-/ml medium)
Dynamic binding capacity (mg/ml medium)†		
BSA (M_r 67 000)	N.D.	> 130
Lysozyme (M_r 14 300)	> 160	N.D

* Water at room temperature. Flow rate is determined by v • η < 10 ml/min where v=flow rate and η=viscosity.

† Determination of dynamic binding capacity: Q Sepharose XL and SP Sepharose XL: Samples were applied at 300 cm/h until 10% breakthrough. Column: 0.75 × 10 cm. Buffers: 0.05 M Tris, (+0.5 M NaCl in the elution buffer), pH 7.5 (Q), 0.05 M glycine, (+0.5 M NaCl in the elution buffer), pH 9.0 (SP).

‡ Many chromatography systems are equipped with pressure gauges to measure the pressure at a particular point in the system, usually just after the pumps. The pressure measured here is the sum of the pre-column pressure, the pressure drop over the gel bed, and the post column pressure. It is always higher than the pressure drop over the bed alone. We recommend keeping the pressure drop over the bed below 1.5 bar. Setting the upper limit of your pressure gauge to 1.5 bar will ensure the pump shuts down before the gel 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 determine post-column pressure, proceed as follows:

To avoid breaking the column, the post-column pressure must never exceed 3.5 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 the post-column pressure as total pressure minus pre-column pressure.
If the 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. When the post-column pressure is satisfactory, add the post-column pressure to 1.5 bar and set this as the upper pressure limit on the chromatography system.

First time use

Ensure an appropriate pressure limit has been set.

Equilibrate the column for first time use or after long storage by running:

1. 100 ml of start buffer (low ionic strength) at 5 ml/min at room temperature (see section "Choice of buffer" for buffer recommendations).
2. 100 ml of elution buffer at 5 ml/min at room temperature.
3. 100 ml of start buffer at 5 ml/min at room temperature.

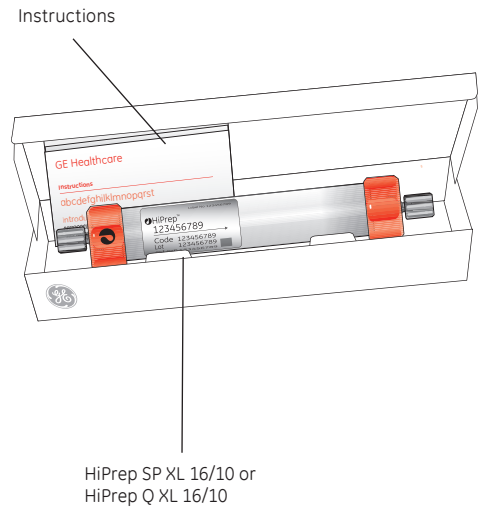
These HiPrep columns can be used directly on ÄKTA™ design systems without the need for any extra connectors.

Try these conditions first

Flow rate:	5 ml/min at room temperature
Start buffer:	See section "Choice of buffer"
Elution buffer:	Start buffer + 1 M NaCl
Gradient:	0–100% elution buffer in 200 ml (10 CV)

Equilibration before a new run

Proceed according to steps 2 and 3 in the section "First time use". Extended equilibration may be needed if detergents were included in the eluent. Please read the back of this instruction for information on optimizing a separation.



Buffer and solvent resistance

De-gas and filter all solutions through a 0.45 µm filter to increase column life-time.

Daily use:

All commonly used aqueous buffers (see "Column data" for recommended pH)

Guanidine hydrochloride, up to 6 M

Urea, up to 8 M

Cleaning:

Sodium hydroxide, up to 1 M

Isopropanol, up to 30%

Avoid:

Oxidizing agents

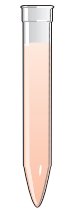
Cationic detergents and buffers (SP)

Anionic detergents and buffers (Q)

Phenol

Sample preparation

Net charge of protein:	Positive (SP), negative (Q)
Recommended sample load:	Not more than 10–20% of the dynamic binding capacity (see "Column data").
Preparation:	Dissolve the sample in start buffer, filter through 0.45 µm or centrifuge at 10 000 × g for 10 min.



Delivery/storage

HiPrep 16/10 Q XL is supplied in 20% ethanol and HiPrep 16/10 SP XL is supplied in 20% ethanol, 0.2 M sodium acetate. If the column is to be stored for more than two days after use, clean the column according to the procedure described under "Cleaning in place (CIP)". Then equilibrate HiPrep 16/10 Q XL with at least 100 ml of 20% ethanol at a flow rate of 5 ml/min at room temperature. Equilibration of HiPrep 16/10 SP XL with 100 ml 20% ethanol, 0.2 M sodium acetate at a flow rate of 5 ml/min at room temperature is recommended.

Note: HiPrep columns cannot be opened or refilled.

Choice of buffer

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

Start buffer pH should be selected so that substances to be bound to the ion exchanger are charged, that is, pH conditions should be 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.

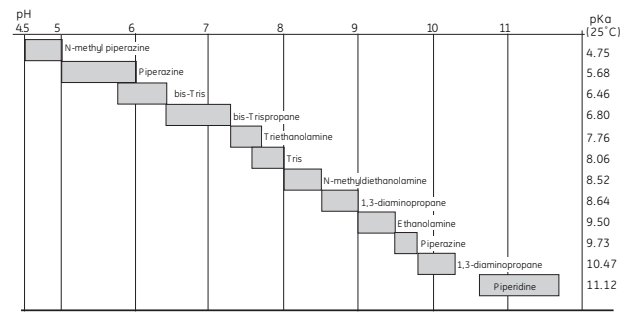


Fig 1. Recommended buffer substances for anion exchange chromatography.

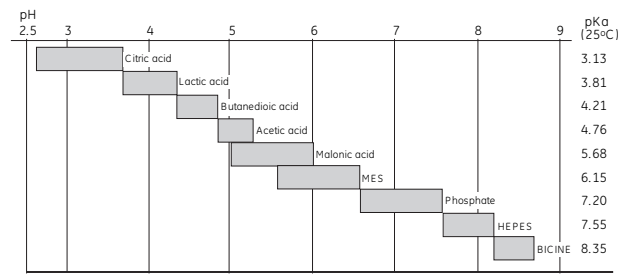


Fig 2. Recommended buffer substances for cation exchange chromatography.

HiPrep

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 your first run according to "Try these conditions first". If the 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
Shallower gradient	Improved resolution, but broader peaks and decreased concentration in fractions

Cleaning-in-place (CIP)

Regular cleaning

Wash the column with 40 ml of 2 M NaCl at a flow rate of 5 ml/min at room temperature after each run to elute material still bound to the column.

If detergents have been used, rinse the column with 100 ml distilled water followed by 40 ml of 2 M NaCl at a flow rate of 5 ml/min at room temperature. Re-equilibrate the column with at least 100 ml start buffer at a flow rate of 5 ml/min at room temperature until the UV baseline and pH/conductivity values are stable.

More rigorous cleaning

Reverse flow direction and run the following sequence of solutions at a flow rate of 5 ml/min at room temperature:

1. 80 ml of a 2 M NaCl solution (removes ionically bound proteins from the column) followed by 50 ml distilled water.
2. 80 ml of a 1 M NaOH solution (removes precipitated proteins, hydrophobically bound proteins, and lipoproteins from the column) followed by 50 ml distilled water.
3. 80 ml of 30% isopropanol (removes proteins, lipoproteins, and lipids that are strongly hydrophobically bound to the column) followed by 60 ml distilled water,
   or
   30 ml 0.5% non-ionic detergent in acidic solution (for example 0.1 M acetic acid) followed by 100 ml 70% Ethanol (to remove the detergent) and 60 ml distilled water.

After cleaning, equilibrate the column with approximately 100 ml start buffer before use at a flow rate of 5 ml/min at room temperature in the normal flow direction.

Note: HiPrep columns cannot be opened or refilled.

Troubleshooting

Symptom	Remedy
Increased backpressure over the column	Reverse the flow direction and pump 100 ml elution buffer at a flow rate of 5 ml/min through the column. Return to normal flow direction and run 100 ml start buffer at a flow rate of 5 ml/min through the column. If back-pressure is not decreased reverse the flow direction again and follow the more rigorous cleaning instruction.
Loss of resolution and/or decreased sample recovery Air in the column	Clean the column according to the procedure described in the section "More rigorous cleaning". Reverse the flow direction and pump 100 ml of well de-gassed start buffer through the column at a flow rate of 5–10 ml/min.

Intended use

The HiPrep SP XL 16/10 and HiPrep Q XL 16/10 are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Ordering information

Product	No. per pack	Code No.
HiPrep 16/10 SP XL	1 × 20 ml	28-9365-40
HiPrep 16/10 Q XL	1 × 20 ml	28-9365-38

Related products	No. per pack	Code No.
HiTrap™ IEX Selection kit*	7 × 1 ml	17-6002-33
HiTrap SP XL	5 × 1 ml	17-5160-01
HiTrap SP XL	5 × 5 ml	17-5161-01
HiTrap Q XL	5 × 1 ml	17-5158-01
HiTrap Q XL	5 × 5 ml	17-5159-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
HiPrep 26/10 Desalting	4 × 53 ml	17-5087-02

Accessories	No. per pack	Code No.
HiTrap/HiPrep 1/16" male connector for ÄKTA design	8	28-4010-81
To connect columns with 1/16" connectors to FPLC™ System: Union M6 female/1/16" male*	5	18-3858-01

Related literature	
Handbook, Ion Exchange Chromatography, and Chromatofocusing, Principles & Methods	11-0004-21
Ion Exchange Chromatography, Media and Column Guide	18-1127-31
Prepacked chromatography columns for ÄKTA design LC systems	28-9317-78

Important information

Separating viral particles with "Q Sepharose XL" products may require a license under United States pat 6.537.793 B2 and foreign equivalents owned by Gencell SAS. Such license is not included with the purchase of this product but is included with the purchase of "Q Sepharose XL virus licensed" products.

Further information

For more information, please visit:

www.gelifesciences.com/protein-purification

www.gelifesciences.com/purification_techsupport

Refer also to the handbook "Ion Exchange Chromatography and Chromatofocusing, Principles and Methods", see ordering information.

www.gelifesciences.com/protein-purification
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