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

Instructions for Use

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.

Table 1. Column data

Matrix	Cross-linked agarose, with dextran surface extender, spherical	
Particle size, d_{50V} [1]	~ 90 µm	
Bed volume	20 mL	
Bed height	100 mm	
i.d.	16 mm	
Column composition	Polypropylene	
Recommended operating flow rate[2]	2–10 mL/min (30 to 300 cm/h)	
Maximum operating flow rate[2]	10 mL/min (300 cm/h	
Maximum pressure over the packed bed during operation, Δp[3]	0.15 MPa, 1.5 bar, 22 psi	
HiPrep column hardware pressure limit[3]	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 stability, operational[4]	4 to 13	2 to 12
pH stability, CIP[5]	3 to 14	2 to 14
pH ligand fully charged[6]	entire pH range	entire pH range
Ionic capacity	0.18–0.25 (mmol H+/mL resin)	0.18–0.26 (mmol Cl-/mL resin)
Dynamic binding capacity, Q_{810} (mg/mL resin)		
BSA (M_r 67 000)	N.D.	≥ 160[7]
Lysozyme (M_r 14 300)	≥ 160[8]	N.D

[1] Median particle size of the cumulative volume distribution.

[2] At room temperature using buffers with the same viscosity as water.

[3] 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 precolumn pressure, the pressure drop over the resin 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 resin 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:

[4] pH range where resin can be operated without significant change in function.

[5] pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

[6] pH range where ligand is fully charged; although the ligand is fully charged throughout the range stated, only use the resin within the stated stability ranges.

[7] Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 300 cm/h in a PEEK 7.5/100 column at 10 cm bed height (2 min residence time) for BSA in 50 mM Tris-HCl, pH 7.5.

[8] Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 300 cm/h in a PEEK 7.5/100 column at 10 cm bed height (2 min residence time) for Lysozyme in 50 mM Glycine-NaOH, pH 9.

To avoid breaking the column, the post-column pressure must never exceed 3.5 bar

Step	Action
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 precolumn pressure.
4	Calculate the post-column pressure as total pressure minus precolumn 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:

Step	Action
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.

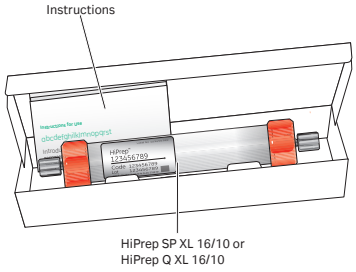
These HiPrep columns can be used directly on ÄKTA™ design systems without the need for any extra connectors.

Try these conditions first

Flow rate:	5 mL/min at room temperature
Start buffer:	See Section *Choice of buffer, on page 2*
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, on page 2*. Extended equilibration may be needed if detergents were included in the eluent. Read the back of this instruction for information on optimizing a separation.



Buffer and solvent resistance

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

Daily use:

Commonly used aqueous buffers (see *Table 1, on page 1* for recommended pH)

Guanidine hydrochloride, up to 6 M

Urea, up to 8 M

Cleaning:

Sodium hydroxide, up to 1.0 M

Avoid:

Oxidizing agents

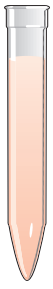
Cationic detergents and buffers (SP)

Anionic detergents and buffers (Q)

Phenol

Sample preparation

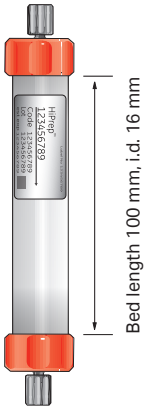
Net charge of protein:	Positive (SP), negative (Q)
Recommended sample load:	Not more than 10–20% of the dynamic binding capacity (see *Table 1, on page 1*).
Preparation:	Dissolve the sample in start buffer, filter through 0.45 µm or centrifuge at 10 000 × g for 10 min.



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), on page 3*. 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 below list a selection of standard aqueous buffers.

Table 2 below lists suggested volatile buffers used in cases where the purified substance has to be freeze-dried.

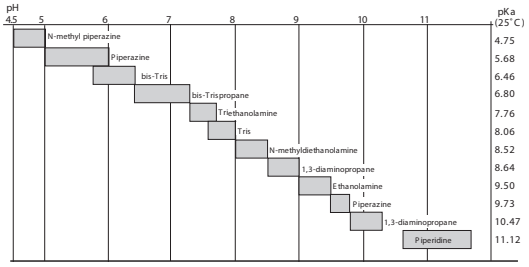


Fig 1. Recommended buffer substances for anion exchange chromatography.

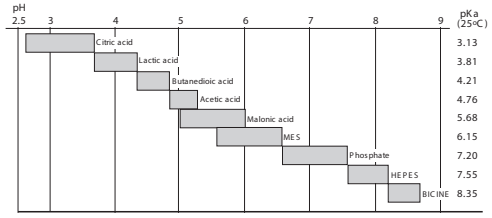


Fig 2. Recommended buffer substances for cation exchange chromatography.

Table 2. 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/HC
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, on page 2*. If the results are unsatisfactory, consider the following:

Action	Effect
Change pH/buffer salt (See Figures 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:

Step	Action
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 1.0 M NaOH solution (removes precipitated proteins, hydrophobically bound proteins, and lipoproteins from the column) followed by 50 mL distilled water.
3	30 mL 0.5% nonionic 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 backpressure 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, on page 3*. Reverse the flow direction and pump 100 mL of well degassed 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	Product code
HiPrep 16/10 SP XL	1 × 20 mL	28936540
HiPrep 16/10 Q XL	1 × 20 mL	28936538

Related products	No. per pack	Product code
HiTrap™ IEX Selection kit*	7 × 1 mL	17600233
HiTrap SP XL	5 × 1 mL	17516001
HiTrap SP XL	5 × 5 mL	17516101
HiTrap Q XL	5 × 1 mL	17515801
HiTrap Q XL	5 × 5 mL	17515901
HiPrep 26/10 Desalting	1 x 53 mL	17508701
HiPrep 26/10 Desalting	4 x 53 mL	17508702

Accessories	No. per pack	Product code
HiTrap/HiPrep 1/16" male connector for ÄKTA design	8	28401081
To connect columns with 1/16" connectors to FPLC System:		
Union M6 female/1/16" male*	5	18385801

Related literature	Product code
Handbook, Ion Exchange Chromatography, Principles & Methods	11000421
Ion Exchange Chromatography, Media and Column Guide	18112731
Prepacked chromatography columns for ÄKTA design LC systems	28931778

Further information

For more information, visit:

cytiva.com/protein-purification

cytiva.com/support

Refer also to the handbook *Ion Exchange Chromatography, Principles and Methods*, see the Section *Ordering information* above.

cytiva.com/protein-purification

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