cytiva

HiScreen Q FF
HiScreen SP FF
HiScreen DEAE FF
HiScreen Q HP
HiScreen SP HP

Prepacked columns

Instructions for Use

HiScreen™ Q FF, HiScreen SP FF, HiScreen DEAE FF, HiScreen Q HP and HiScreen SP HP are prepacked, ready-to-use ion exchange chromatography (IEX) columns. The prepacked 4.7 mL columns are ideal for screening of selectivity, binding and elution conditions, as well as small scale purifications.

These HiScreen IEX columns prepacked with Q Sepharose™ Fast Flow (FF), SP Sepharose Fast Flow, DEAE Sepharose Fast Flow, Q Sepharose High Performance (HP) and SP Sepharose High Performance provide fast, reproducible and easy separations in a convenient format. The columns are used in an optimal way with liquid chromatography systems such as ÄKTA™.

Table of Contents

Read these instructions carefully before using the products.

Intended use

The products 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 products in a safe way, refer to the Safety Data Sheets.

1 Product description

HiScreen column characteristics

HiScreen columns are made of biocompatible polypropylene that does not interact with biomolecules. The arrow on the column label shows the recommended flow direction .

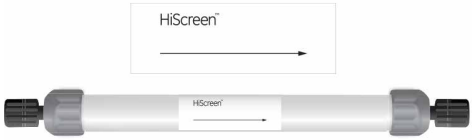

Fig 1. HiScreen column

For scale-up, when a higher bed height is required, two columns can be connected in series using a union to give a 20 cm bed height (see Section *Scaling up*).

Note: *Do not open or refill HiScreen columns.*

Note: *Check that the connector is tightened to prevent leakage.*

Table 1. Characteristics of HiScreen column

Column volume (CV)	4.7 mL
Column dimensions	0.77 × 10 cm
Column hardware pressure limit	0.8 MPa (8 bar, 116 psi)

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

Properties of IEX chromatography resins

SP Sepharose and Q Sepharose are strong cation and strong anion exchangers, respectively. The functional groups are coupled to the matrix via chemically stable ether linkages and remain charged over the entire pH working range, as well as maintain high capacity.

- Q: strong anion exchanger (quaternary amine group)
- SP: strong cation exchanger (sulfoethyl group)
- DEAE: weak anion exchanger (diethylaminoethyl group)

The characteristics of the different resins are listed in Table 2 and 3.

Q Sepharose Fast Flow is a strong anion exchanger based on 6% cross-linked agarose with an average bead size of 90 µm. This resin has good flow properties and high loading capacities.

SP Sepharose Fast Flow is a strong cation exchanger based on 6% cross-linked agarose with a particle size of ~ 90 µm. This resin has good flow properties and high loading capacities.

DEAE Sepharose Fast Flow is a weak anion exchanger based on 6% cross-linked agarose with a particle size of ~ 90 µm. This resin has good flow properties and high loading capacities.

Q Sepharose High Performance is a strong anion exchanger based on rigid, cross-linked agarose with a particle size of ~ 34 µm. The smaller bead size will result in higher resolution and sharper peaks.

SP Sepharose High Performance is a strong cation exchanger based on rigid, cross-linked agarose with a particle size of ~ 34 µm. The smaller bead size will result in higher resolution and sharper peaks.

Table 2. Characteristics of different Sepharose FF resins

	Q Sepharose FF	SP Sepharose FF	DEAE Sepharose FF
Matrix	Cross-linked agarose, 6%, spherical		
Particle size d_{50v} [1]	~ 90 µm	~ 90 µm	~ 90 µm
Ion exchange type	Strong anion	Strong cation	Weak anion
Charged group	$-N^+(CH_3)_3$	$-CH_2CH_2CH_2SO_3^-$	$-N^+(C_2H_5)_2H$
Ionic capacity	0.18 to 0.25 mmol Cl^-/mL resin	0.18 to 0.25 mmol H^+/mL resin	0.11 to 0.16 mmol Cl^-/mL resin
Dynamic binding capacity [2]	~ 42 mg BSA/mL resin	~ 70 mg Ribonuclease A/mL resin	~ 110 mg HAS/mL resin
Recommended operating flow rate (HiScreen) [3]	300 cm/h (2.3 mL/min)	300 cm/h (2.3 mL/min)	300 cm/h (2.3 mL/min)
Maximum operating flow rate (HiScreen) [3]	450 cm/h (3.5 mL/min)	450 cm/h (3.5 mL/min)	450 cm/h (3.5 mL/min)
pH stability, operational [4]	2 to 12	4 to 13	2 to 12
pH stability, CIP [5]	2 to 14	3 to 14	2 to 14
pH ligand fully charged	Entire operational pH range		Below 9[6]
Chemical stability	Stable to commonly used aqueous buffers, 1 M NaOH[7], 8 M urea, 6 M guanidine hydrochloride, 70% ethanol		
Storage	20% ethanol, 4°C to 30°C	0.2 M sodium acetate in 20% ethanol, 4°C to 30°C	20% ethanol, 4°C to 30°C

1 Median particle size of the cumulative volume distribution.

2 Running conditions: Q Sepharose FF and DEAE Sepharose FF: 0.05 M Tris-HCl, pH 7.5 at 75 cm/h (DEAE) and 300 cm/h (Q). SP Sepharose FF: 0.1 M sodium acetate, pH 5.0 at 75 cm/h.

3 At room temperature using buffers with the same viscosity as water.

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] 1.0 M NaOH should only be used for cleaning purposes.

Table 3. Characteristics of different Sepharose HP resins

	Q Sepharose HP	SP Sepharose HP
Matrix	Cross-linked agarose, spherical	
Particle size (d_{50v}) [1]	~ 34 µm	~ 34 µm
Ion exchange type	Strong anion	Strong cation
Charged group	$-N^+(CH_3)_3$	$-CH_2CH_2CH_2SO_3^-$
Ionic capacity	0.14 to 0.20 mmol Cl^-/mL resin	0.15 to 0.20 mmol H^+/mL resin
Dynamic binding capacity [2]	~ 70 mg BSA/mL resin	~ 55 mg Ribonuclease A/mL resin
Recommended operating flow rate (HiScreen) [3]	30 to 150 cm/h (0.6 mL/min)	30 to 150 cm/h (0.6 mL/min)
Maximum operating flow rate (HiScreen) [3]	150 cm/h (1.2 mL/min)	150 cm/h (1.2 mL/min)
pH stability, operational [4]	2 to 12	4 to 13
	2 to 14	3 to 14
pH stability, CIP [5]	Entire operational pH range	Entire operational pH range
pH ligand fully charged		
Chemical stability	Stable to commonly used aqueous buffers, 1.0 M NaOH[6], 8 M urea, 6 M guanidine hydrochloride, 70% ethanol	
Storage	20% ethanol, 4°C to 30°C	0.2 M sodium acetate in 20% ethanol, 4°C to 30°C

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

[2] Running conditions: Q Sepharose HP: 0.05 M Tris-HCl, pH 8 at 150 cm/h. SP Sepharose HP: 0.1 M sodium acetate, pH 6.0 at 156 cm/h.

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

[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] 1.0 M NaOH should only be used for cleaning purposes.

2 General process development

HiScreen column format is ideal to use for parameter and method optimization and for robustness testing when developing a new purification process. The small column volume, 4.7 mL, and the 10 cm bed height makes it possible to perform scalable experiments at relevant process flow rates. If necessary, two columns can easily be connected in series with a union to give 20 cm bed height (see Section *Scaling up*).

The figure below outlines typical steps during general process development.

Already from start in process development it is necessary to consider process cost, cleaning of the resin, and environmental constraints.

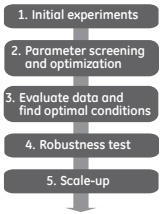

Fig 2. Typical steps during process development.

Design of Experiments (DoE) is an effective tool for investigating the effect of several parameters on protein recovery in order to establish the optimal purification protocol. See handbook *Design of Experiments in Protein Production and Purification*.

A common approach in DoE is to define a reference experiment (center point) and perform representative experiments around that point. Some initial experiments are required in order to define the center point and the variable ranges. DoE can be used for parameter screening and optimization as well as robustness testing.

The robustness of a process is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters, and provides an indication of its reliability during normal usage. An objective of a robustness test is the evaluation of factors potentially causing variability in the responses of the method, for example, purity and yield. For this purpose, small variations in method parameters are introduced.

For scale-up, see Section *Scaling up*.

3 Optimization

Optimizing the process

The aim of designing and optimizing an ion exchange separation process is to identify conditions that promote binding of the highest amount of target molecule in the shortest possible time with highest possible product recovery. To reduce time, sample and buffer consumption during optimization the method should be designed in laboratory scale.

Optimizing binding conditions

Screen for optimal binding conditions by testing a range of pH values within which the target protein is known to be stable. If the isoelectric point (pI) of the target protein is known, then begin with a narrower pH range, for example, 0.5 to 1 pH unit away from pI. In some cases the sample conductivity is equally important as the pH when screening for optimal binding conditions.

For certain proteins, dynamic binding capacities increase at increased conductivity. Our recommendation is to screen for optimal ionic strength by varying the conductivity of the sample between
2 and 15 mS/cm as well as screening for optimal binding pH.

Optimizing elution conditions

Linear ionic strength gradients should always be used for method development or when starting with an unknown sample. Linear ionic strength gradients are easy to prepare and very reproducible when generated by a suitable chromatography system. The results obtained can then serve as a basis from which to optimize the separation.

Step-wise elution allows the target protein to be eluted in a more concentrated form, thus decreasing buffer consumption and shortening cycle times. Due to the high concentrations of protein in the eluted pool it might be necessary to decrease the flow rate and thereby avoid exceeding the maximum back pressure for the column.

Automated buffer preparation

Users of ÄKTA chromatography systems with BufferPrep or BufferPro functionality can select from a range of buffer recipes to conveniently screen resins over a range of pH values and elution conditions.

4 Operation

Prepare buffers

The elution buffer is usually of the same composition and pH as the start buffer, but it contains additional salt, most often sodium chloride. The pH of the start buffer should be at least 0.5 to 1 pH unit above the isoelectric point (pI) of the target molecule when using an anion exchanger (Q and DEAE) and at least 0.5 to 1 pH unit below the pI when using a cation exchanger (SP).

The buffer species and buffer concentration are important for reproducible and robust methods. The buffer concentration depends partly on the buffer capacity at a given pH and should be at least 10 mM (only rarely above 100 mM). Where the conductivity of the buffers needs to be considered, it can be increased by increasing the buffer concentration or adding sodium chloride.

Try the following buffers for samples with unknown charge properties.

Anion exchange (Q and DEAE)

Start buffer: 20 mM Tris-HCl, pH 8.0

Elution buffer: 20 mM Tris-HCl, 1 M NaCl, pH 8.0

Cation exchange (SP)

Start buffer: 50 mM sodium acetate, pH 5.0

Elution buffer: 50 mM sodium acetate, 1 M NaCl, pH 5.0

or

Start buffer: 50 mM MES, pH 6.0

Elution buffer: 50 mM MES, 1 M NaCl, pH 6.0

Note: *Water and chemicals used for buffer preparation should be of high purity. Filter buffers through a 0.22 µm or a 0.45 µm filter before use.*

Prepare the sample

Step	Action
1	Adjust the sample to the composition of the start buffer, using one of these methods: • Dilute the sample with start buffer. • Exchange buffer using a HiPrep™ 26/10 Desalting, HiTrap™ Desalting or PD-10 Desalting column (see table below).
2	Filter the sample through a 0.45 µm filter or centrifuge immediately before loading it to the column. This prevents clogging and increases the life time of the column when loading large sample volumes.

Table 4. Prepacked desalting columns

Column	Loading volume	Elution volume
HiPrep 26/10 Desalting[1]	2.5 to 15 mL	7.5 to 20 mL
HiTrap Desalting[2]	0.25 to 1.5 mL	1.0 to 2.0 mL
PD-10 Desalting[3]	1.0 to 2.5 mL[4]	3.5 mL
	1.75 to 2.5 mL[5]	Up to 2.5 mL
PD MiniTrap™ G-25	0.1 to 2.5 mL[4]	1.0 mL
	0.2 to 0.5 mL[5]	Up to 0.5 mL
PD MidiTrap™ G-25	0.5 to 1 mL[4]	1.5 mL
	0.75 to 1 mL[5]	Up to 1 mL

[1] Prepacked with Sephadex™ G-25 Fine and requires a pump or a chromatography system to run.

[2] Prepacked with Sephadex G-25 Superfine and requires a syringe or pump to run.

[3] Prepacked with Sephadex G-25 and can be run by the gravity flow or centrifugation.

[4] Volumes with gravity elution.

[5] Volumes with centrifugation.

Purification

Collect fractions throughout the separation.

Flow rate: See Tables 2 and 3.

Column tubing: Choose the optimal tubing kit for the column and the application you intend to run. (i.d.: 0.25, 0.50 or 0.75 [mm]). A tubing with wider inner diameter gives broader peaks whereas a tubing with a smaller inner diameter gives higher back pressure.

Step	Action
1	Remove the stoppers and connect the column to the system. Avoid introducing air into the column. ***Note:*** *To prevent leakage, make sure that the connectors are tight. Use fingertight 1/16" connector (28401081).*
2	Wash with 1 column volume (CV) of distilled water. This step removes the ethanol and avoids the precipitation of buffer salts upon exposure to ethanol. The step can be omitted if precipitation is not likely to be a problem.
3	Adjust the sample to the chosen starting pH and conductivity and load on the column.
4	Wash with 5 to 10 CV start buffer or until the UV trace of the effluent returns to near baseline.

Step	Action
5	• *Linear gradient elution* Elute with 0 to 100% elution buffer (up to 1 M NaCl) in 10 to 20 CV. • *Step elution* Elute with 5 CV elution buffer including NaCl at chosen concentration. Repeat at higher NaCl concentrations until the target protein has been eluted
6	Wash with 5 CV 1 M NaCl (100% elution buffer) to elute any remaining ionically bound material.
7	If required, perform a CIP to clean the column.
8	Re-equilibrate with 5 to 10 CV start buffer or until the UV baseline, eluent pH, and conductivity reach the required values.

To save time, higher flow rates during regeneration and re-equilibration steps can be used.

Note: *Do not exceed the maximum recommended flow and back pressure for the column.*

5 Cleaning-in-place (CIP)

General description

Correct preparation of samples and buffers maintains columns in good condition. However, reduced performance, increased back pressure or blockage indicates that the column needs cleaning.

CIP removes very tightly bound, precipitated, or denatured substances from the resin. If such contaminants are allowed to accumulate, they can affect the chromatographic properties of the prepacked column, reduce the capacity of the resin and, potentially, come off in subsequent runs. If the fouling is severe, it can block the column, increase back pressure, and affect the flow properties.

CIP must be performed regularly to prevent the build-up of contaminants and to maintain the capacity, flow properties, and general performance of prepacked columns.

It is recommended to perform a CIP:

- When an increase in back pressure is observed.
- If reduced column performance is observed.
- Between runs when the same column is used for purification of different proteins to prevent possible cross-contamination.
- Before first-use or after long-term storage.
- After every run with real feed.

CIP protocol

The nature of the sample will ultimately determine the final CIP protocol so the CIP procedure below may require optimization. NaOH concentration, contact time and frequency are typically the main parameters to vary during the optimization of the CIP.

The CIP procedure below removes common contaminants.

Flow rate: For increased contact time and due to the viscosity of the CIP solutions it is recommended to use a lower flow rate than during purification.

Regular cleaning

Step	Action
1	Wash with 2 column volumes (CV) 2 M NaCl. If detergents have been used, wash with 5 CV distilled water followed by 2 CV of 2 M NaCl.
2	Re-equilibrate the column with at least 5 CV start buffer or until the UV baseline and pH/conductivity values are stable.

More rigorous cleaning

Reverse the flow direction and use the following sequence of solutions.

Step	Action
1	4 CV 2 M NaCl (removes ionically bound proteins) followed by 2 CV distilled water.

Step	Action
2	4 CV 1.0 M NaOH (removes precipitated proteins, hydro-phobically bound proteins, and lipoproteins) followed by 4 CV distilled water.
3	2 CV 30% isopropanol (removes proteins, lipoproteins, and lipids that are strongly hydrophobically bound) followed by 4 CV distilled water. or 2 CV 0.5% nonionic detergent in acidic solution (e.g., 0.1 M acetic acid) followed by 5 CV 70% ethanol (to remove the detergent) followed by 3 CV distilled water.

After cleaning, equilibrate the column before use with approximately 5 CV start buffer in the normal flow direction.

6 Scaling up

After optimizing the method at laboratory-scale, the process is ready for scaling up.

For quick scale-up of purification, two HiScreen columns can easily be connected in series with a union (18112093) to give 20 cm bed height.

Note: *The back pressure is increased with longer bed height. This is easily addressed by lowering the flow rate.*

Other factors, such as the clearance of critical impurities can change, when column bed height is modified. The factors must be validated using the final bed height.

Scale-up to a larger column is typically performed by keeping bed height and flow velocity (cm/h) constant while increasing bed diameter and flow rate (mL/min or L/h).

7 Adjusting pressure limits

The pressure in chromatography system software is generated by the flow through a column. The pressure affects the packed bed and the column hardware, see the figure below. The pressure is increased during 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 recommended flow rates in Table 2, on page 5 and Table 3, on page 6) can damage the column.*

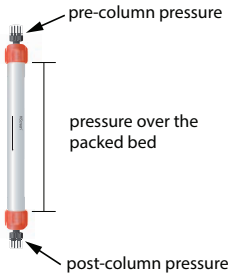

Fig 3. Precolumn and post-column measurements.

ÄKTA avant and ÄKTA pure

The system will automatically monitor the pressures (precolumn pressure and pressure over the packed bed, Δp). The precolumn pressure limit is the column hardware pressure limit (see *Table 1, on page 3* and *Table 2, on page 5*).

The maximum pressure the packed bed can withstand depends on resin 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 the optimal functionality in ÄKTAexplorer, ÄKTApurifier, ÄKTAFPLC, and other systems with pressure sensor in the pump, the pressure limit in the software can be adjusted as follows:

Step	Action
1	• Replace the column with a piece of tubing. • Run the pump at the maximum intended flow rate. • Record 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. • Record the 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. *Result:* The actual pressure over the packed bed (Δp) during the run is equal to the actual measured pressure which is the total system pressure (P1).

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

8 Storage

HiScreen Q FF, HiScreen DEAE FF and HiScreen Q HP : Wash with 2 column volumes (CV) of distilled water followed by 2 CV of 20% ethanol.

HiScreen SP FF and HiScreen SP HP : Wash with 2 CV of distilled water followed by 2 CV of 20% ethanol containing 0.2 M sodium acetate.

Store at 4°C to 30°C. Do not freeze.

Make sure that the column is tightly sealed to avoid drying out.

9 Troubleshooting

Problem	Possible cause	Corrective action
High back pressure during the run	Solutions with high viscosity are used.	Use a lower flow rate.
	The column is clogged	Clean the column, see section Cleaning-in-place (CIP)
Unstable pressure curve during sample loading	Air bubbles trapped in the sample pump.	If possible, degas the sample using a vacuum degasser.
Gradual broadening of the eluate peak	Insufficient elution and CIP, caused by contaminants accumulating in the column.	Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.
Gradual decrease in yield	Insufficient elution and CIP.	Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.
Precipitation during elution	Suboptimal elution conditions and CIP.	Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.
Gradual increase in CIP peaks	Suboptimal elution conditions and CIP.	Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.
High back pressure during CIP	Proteins are precipitated in the column.	Optimize elution conditions and/or run high salt wash before CIP or use lower flow rate.

Problem	Possible cause	Corrective action
Reduced column performance despite optimized elution and CIP	Column longevity, which depends mainly on the sample type and sample preparation.	Change to a new column.

10 Ordering information

Product	Quantity	Product code
HiScreen Q FF	1 × 4.7 mL	28950510
HiScreen SP FF	1 × 4.7 mL	28950513
HiScreen DEAE FF	1 × 4.7 mL	28978245
HiScreen Q HP	1 × 4.7 mL	28950511
HiScreen SP HP	1 × 4.7 mL	28950515

Related products	Quantity	Product code
HiTrap IEX Selection Kit, 7 different IEX resins	7 × 1 mL	17600233
HiTrap Q FF	5 × 1 mL	17505301
	5 × 5 mL	17515601
HiTrap SP FF	5 × 1 mL	17505401
	5 × 5 mL	17515701
HiTrap DEAE FF	5 × 1 mL	17505501
	5 × 5 mL	17515401
HiTrap SP HP	5 × 1 mL	17115101
	5 × 5 mL	17115201
HiTrap Q HP	5 × 1 mL	17115301
	5 × 5 mL	17115401
HiPrep Q FF 16/10	1 × 20 mL	28936543
HiPrep SP FF 16/10	1 × 20 mL	28936544
HiPrep DEAE FF 16/10	1 × 20 mL	28936541

Related products	Quantity	Product code
Q Sepharose Fast Flow	25 mL	17051010
	300 mL[1]	17151001
SP Sepharose Fast Flow	25 mL	17072910
	300 mL[1]	17072901
DEAE Sepharose Fast Flow	25 mL	17070910
	500 mL[1]	17070901
Q Sepharose High Performance	75 mL	17101401
	1 L[1]	17101403
SP Sepharose High Performance	75 mL	17108701
	1 L[1]	17108703
HiTrap Desalting	5 × 5 mL	17140801
HiPrep 26/10 Desalting	1 × 53 mL	17507801
	4 × 53 mL	17508702

[1] Process-scale quantities are available. Please contact your local representative.

Accessories HiScreen	Quantity	Product code
HiTrap/HiPrep, 1/16" male connector for ÄKTA *(For connection of columns with 1/16" fittings to ÄKTA systems)*	8	28401081
Union 1/16" male/1/16" male with 0.5 mm i.d. *(For connecting two columns with 1/16" fittings in series)*	2	18112093
Fingertight stop plug, 1/16"[1] *(For sealing a HiScreen column)*	5	11000355

[1] One fingertight stop plug is connected to the inlet and the outlet of each HiScreen column at delivery.

Related literature	Product code
Ion Exchange Chromatography Handbook, Principles and Methods	11000421
Ion Exchange Columns and Media, Selection Guide	18112731
Prepacked chromatography columns for ÄKTA systems, Selection Guide	28931778

Page intentionally left blank

cytiva.com/protein-purification

For local office contact information, visit cytiva.com/contact

28950367 AF V:5 06/2020