Q Sepharose™ High Performance

Ion exchange chromatography resin

Instructions for Use

Q Sepharose™ High Performance is a BioProcess™ anion ion exchange chromatography resin intended for purifying a wide range of biomolecules.

The resin has the following characteristics:

- high-resolution, high-capacity separations with high recovery
- reliable and reproducible
- high chemical stability for effective Cleaning-In-Place (CIP)/sanitization
- available in various convenient prepacked formats, such as PreDictor™ 96-well filter plates, PreDictor RoboColumn units, as well as HiTrap™, HiScreen™, and HiPrep™ columns
- easy to scale up

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Read these instructions carefully before using the product.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

1 BioProcess resins

BioProcess chromatography resins are developed and supported for production-scale chromatography. All BioProcess resins are produced with validated methods and are tested to meet manufacturing requirements. Secure ordering and delivery routines give a reliable supply of resins for production-scale. Regulatory Support Files (RSF) are available to assist process validation and submissions to regulatory authorities. BioProcess resins cover all purification steps from capture to polishing.

2 Resin properties

Q Sepharose High Performance is a strong anion exchanger based on rigid, cross-linked, beaded agarose with a particle size of ~ 34 µm. The ion exchange group is a quaternary amine, see the figure below, which remains charged and maintains consistent ionic capacity over the entire operating range, pH 2–12. The ionic exchange groups are coupled to the base matrix through chemically stable ether bonds.

Fig 1. Partial structure of Q Sepharose High Performance.

The high ionic capacity of Q Sepharose High Performance is illustrated by the titration curve in the figure below. The ionic capacity is 0.14 to 0.20 mmol/mL resin. Dynamic capacity is in the range 50 to 100 mg/mL resin, for example, binding capacity for bovine serum albumin (BSA) is ~ 70 mg/mL resin (column diameter: 5 mm, bed height: 10 cm, sample concentration: 10 mg BSA/mL, and buffer: 50 mM Tris-HCl).

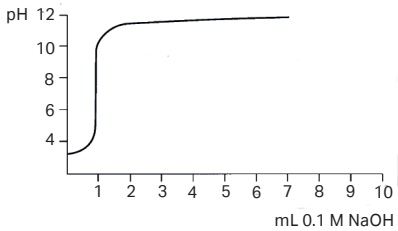

Fig 2. Titration curve. Approx. 5 mL of Q Sepharose High Performance resin in 50 mL 1 M HCl.

Q Sepharose High Performance has the physical and chemical stability to withstand the conditions used in process hygiene procedures, see *Table 1, on page 6*. Strong oxidizing agents, however, must be avoided. The resin combines fast kinetics with physical stability and can therefore perform at flow velocities up to 90 cm/h in columns with 20 cm bed height. The pressure-flow curve is shown in the figure below.

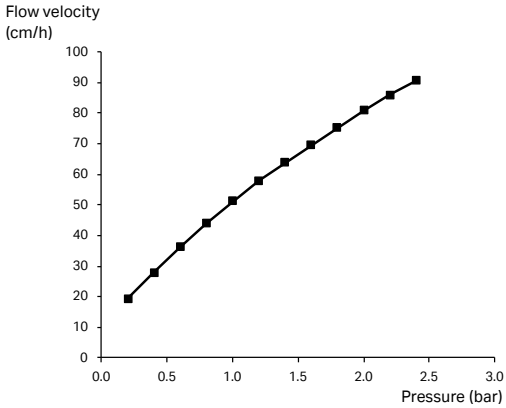

Fig 3. Pressure-flow curve for Q Sepharose High Performance in a 10 L ReadyToProcess™ column with 25 cm diameter, 20 cm bed height using buffers with the same viscosity as water at 20°C.

Table 1. Characteristics of Q Sepharose High Performance

Matrix	Cross-linked agarose, spherical
Functional group	$-CH_2N^+(CH_3)_3$, quaternary ammonium
Ionic capacity	0.14 to 0.20 mmol (Cl^-)/mL resin
Particle size, d_{50v}[1]	~ 34 µm
Recommended operational flow velocity	90 cm/h[2]
Dynamic binding capacity, $QB_{10\%}$[3]	~ 70 mg BSA/mL resin
pH stability, operational[4]	2 to 12
pH stability, CIP[5]	2 to 14
pH ligand fully charged[6]	Entire pH range
Working temperature	4°C to 30°C
Chemical stability	Stable in commonly used aqueous buffers, 1.0 M NaOH[7], 1.0 M acetic acid, 8 M urea, 6 M guanidine hydrochloride, 30% acetonitrile, 30% isopropanol, 70% ethanol and 2% SDS
Avoid	Oxidizing agents, anionic detergents
Storage	20% ethanol, 4°C to 30°C

1 Median particle size of the cumulative volume distribution.

2 In a 10 L ReadyToProcess column with 25 cm diameter and 20 cm bed height using buffer with the same viscosity as water at 20°C.

3 Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 150 cm/h in a PEEK 4.6/50 column at 5 cm bed height (2 min residence time) for BSA in 50 mM Tris-HCl, pH 8.0.

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 entire pH range, only use the resin within the stated stability ranges.

7 1.0 M NaOH should only be used for cleaning purposes.

3 Method optimization

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 and purity. Method development is done at laboratory scale.

For certain proteins, dynamic binding capacities increase at increased conductivity and this is pH dependent. Therefore, scouting of both pH and conductivity for optimal dynamic binding conditions on Q Sepharose High Performance is recommended. Flow velocity can also be included in the scouting.

Elution of protein can either be done by use of salt, pH or a combination of both. For optimization of the elution, sample load, flow velocity and gradient volume should be considered. The three factors are interrelated and best results will be obtained using:

- maximized sample load with respect to dynamic binding capacity
- maximized flow velocity with respect to system constraints and resin rigidity
- the gradient volume that provides the best resolution with maximized sample load and maximized flow velocity

Initial screening of binding and elution conditions can typically be performed using PreDictor plates. PreDictor plates are 96-well filter plates prefilled with chromatography resins, which can be used for rapid screening of chromatographic conditions at small scale. The suggested workflow with PreDictor plates is shown in *Fig. 4, on page 8*, where a large design space can be explored prior to further experiments in packed column formats, such as prepacked HiScreen columns.

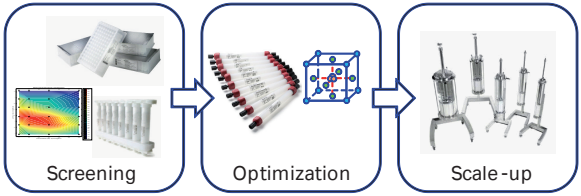

Fig 4. The recommended workflow is described in the figure. It starts with screening of conditions in high-throughput formats, followed by optimization, preferably by applying Design of Experiments (DoE), in small columns, and finally scale-up to large columns.

Table 2.

Phases	Activity	Conditions to consider
1. Equilibration of column and sample preparation	Equilibration of column and adjustment of sample	• pH • Conductivity • Column volume • Column bed height • Particle content • Temperature
2. Sample application	Manual or automatic application onto the column	• Flow rate • Sample pH • Sample conductivity • Upflow/downflow
3. Wash	Wash out unbound material with clean binding buffer	• Flow rate • Upflow/downflow • Buffer choice (normally same as column equilibration buffer)
4. Elution	Elute the material from the column either with salt or by change in pH	• Sample load • pH • Conductivity • Flow rate • Upflow/downflow

For more information about method development and optimization, refer to the handbook *Ion exchange Chromatography: Principles and Methods (CY13983)*.

4 Scale-up

After optimizing the method at laboratory scale, the process can be scaled up. Scale-up is typically performed by keeping bed height and flow velocity constant while increasing bed diameter and volumetric flow rate. However, since optimization is preferably performed with small column volumes, in order to save sample and buffer, some parameters such as the dynamic binding capacity can be optimized using shorter bed heights than those being used at the final scale. As long as the residence time is kept constant, the binding capacity for the target molecule remains the same.

Other factors, such as clearance of critical impurities, can change when column bed height is modified and must be validated using the final bed height. The residence time is calculated as the bed volume (mL) divided by the volumetric flow rate (mL/min) applied during sample loading.

Procedure

Step	Action
1	Select bed volume according to required binding capacity. Keep sample concentration and gradient slope constant.
2	Select column diameter to obtain the bed height (10 to 40 cm) from method optimization. ***Note:*** *The excellent rigidity of the high-flow base matrix allows for flexibility in choice of bed heights.*

Step	Action
3	The use of larger equipment might cause deviations from the method optimized at small scale. In such cases, check the buffer delivery and monitoring systems for time delays or volume changes. Different lengths and diameters of outlet tubing can cause zone spreading on larger systems. Check also the compatibility of the hardware and chromatography resin pressure limits with expected pressure during packing and operation.

5 Column packing

Packing HiScale™ and XK columns

The following instructions are for packing HiScale 10/40, HiScale 16/20, HiScale 26/20, XK 16/20, and XK 26/20 columns with 10 cm bed height.

For more details about packing HiScale columns, refer to instructions *HiScale columns (10, 16, 26, 50) and accessories (28967470)*.

For more details about packing XK columns, refer to *Instruction (28992023)*.

Recommended columns	Inner diameter (mm)	Bed volume	Bed height (cm)
AxiChrom™	50–200	up to 16.7 L	max. 50
AxiChrom	300–1600	up to 1005 L	max. 50
Tricorn™ 5/20	5	up to 0.5 mL	max. 2.6
Tricorn 5/50	5	0.2–1.1 mL	0.8–5.6
Tricorn 10/20	10	up to 2.1 mL	max 2.6
Tricorn 10/50	10	up to 4.4 mL	max. 5.6
Tricorn 10/100	10	3.6–8.4 mL	4.6–10.6
HiScale 10/40	10	8–31 mL	10–40
HiScale 16/20	16	up to 40 mL	0–20
HiScale 16/40	16	16–80 mL	8–40
HiScale 26/20	26	up to 106 mL	0–20
HiScale 26/40	26	69–212 mL	13–40
HiScale 50/20	50	up to 393 mL	0–20
HiScale 50/40	50	274–785 mL	14–40
XK 16/20	16	up to 31 mL	max. 15.5
XK 16/40	16	17–70 mL	8–35
XK 26/20	26	up to 66 mL	max. 12.5
XK 26/40	26	45–186 mL	8.5–35

Materials needed

- Q Sepharose High Performance
- HiScale column or XK column
- HiScale packing tube
- plastic spoon or spatula
- glass filter funnel G4
- vacuum suction equipment
- filter flask
- measuring cylinder
- distilled water

Equipment

- a chromatography system such as ÄKTA™ system or a stand-alone pump, depending on the flow rate required
- a pressure monitor

Equilibrate all materials to room temperature.

Preparation of the slurry

To measure the slurry concentration, let the resin settle in 20% ethanol at least overnight in a measuring cylinder or use the Slurry Concentration Kit (29096100). This method can also be used for HiScale and XK columns.

Washing the resin

Attach a glass filter funnel to a filter flask. Shake the resin to suspend it, pour it into the funnel, and wash it as follows:

Step	Action
1	Wash 5 times with 1 to 2 mL distilled water/mL resin.
2	Gently stir with a spatula between additions.
3	Transfer the resin from the funnel to a beaker and add distilled water to obtain a 50% slurry concentration.

Packing preparations

Step	Action
1	Attach the packing reservoir at the top of the column and rinse with distilled water.
2	Assemble filter and bottom piece on the column.
3	Wet the bottom filter by injecting 20% ethanol through the effluent tubing.
4	Attach the column and packing reservoir vertically on a laboratory stand. Rinse them with distilled water.
5	Apply distilled water 2 cm over the column end piece and put a tubing clamp on the effluent tubing.
6	Pour all the separation resin slurry into the column and packing reservoir and top up carefully with distilled water.

Packing procedure

Step	Action
1	Connect the pump outlet to the inlet on the packing reservoir and open the column outlet.
2	Pack the column with distilled water at a constant flow (see *Table 3, on page 16*, Step 1) until the resin bed is stable.
3	Adjust the flow rate to twice the final one (see Table below, Step 2) and gradually decrease it until the pressure signal is 480 ± 20 kPa. Pack the column at the flow rate which gives 480 ± 20 kPa for 45 minutes.
4	Detach the packing reservoir.
5	Carefully fill the rest of the column with distilled water to form an upward meniscus at the top and insert the flow adapter. The adapter must be adjusted down to the bed surface.
6	Continue packing the column at 480 ± 20 kPa for 6 minutes.
7	Mark on the column the position of the bed surface.
8	Stop the pump.
9	Close the column outlet and adjust the adapter to the bed surface.
10	Lower the adapter a further 3 mm into the bed.

Note: *It is recommended to perform CIP after packing, as column packing involves open handling of the resin.*

Table 3. Packing parameters

Column	Sedimented[1] resin (mL)	Slurry (mL)	Height (mm)	Step 1 (mL/min)	Step 2 (kPa)	Final flow rate (mL/min)
HiScale 10/40	10	20	100	0.4	480 ± 20	~ 5
HiScale or XK 16/20	25	50	100	1.0	480 ± 20	~ 12
HiScale or XK 26/20	66	132	100	2.5	480 ± 20	~ 30

[1] Sedimented resin volume = 1.25 × Packed resin volume.

6 Evaluation of packed column

Introduction

Packing quality needs to be checked by column efficiency testing. The test must be done after packing, at regular intervals during the working life of the column, and also when separation performance has deteriorated.

Column efficiency testing

The best method of expressing the efficiency of a packed column is in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor (A_s). The values are easily determined by applying a test sample such as 1% acetone solution or sodium chloride to the column.

Note: *Use a concentration of 0.8 M NaCl in water as sample and 0.4 M NaCl in water as eluent.*

The calculated plate number depends on the test conditions and must only be used as a reference value. It is important that the test conditions and the equipment are the same so that the results are comparable.

Note: *Changing the solute, solvent, eluent, sample volume, flow velocity, liquid pathway, temperature, chromatography system, etc., influences the results.*

For more information about column efficiency testing, refer to the application note *Column efficiency testing (CY13149)*.

For optimal column efficiency results, the sample volume must be approximately 1% of the column volume and the flow velocity 30 cm/h. If an acceptance limit is defined in relation to column performance, the column plate number can be used as one of the acceptance criteria for the column use.

Method for measuring HETP and A_S

Calculate HETP and A_S from the UV curve (or conductivity curve) as follows:

$$HETP = \frac{L}{N}$$

L = bed height (cm)

N = number of theoretical plates

$$N = 5.54 \times \left(\frac{V_R}{W_h}\right)^2$$

V_R = volume eluted from the start of sample application to the peak maximum

W_h = peak width measured as the width of the recorded peak at half of the peak height

V_R and W_h are in the same units.

The concept of reduced plate height is often used for comparing column performance.

The reduced plate height (h) is calculated as follows:

$$h = \frac{HETP}{d_{50v}}$$

d_{50v} = median particle size of the cumulative volume distribution (cm)

As a guideline, an acceptance value of < 3 for (h) can be used.

The peak must be symmetrical, and the asymmetry factor as close to 1 as possible. A typical acceptable range is $0.8 < A_S < 1.5$.

A change in the shape of the peak is usually the first indication of bed deterioration due to excessive use.

Peak asymmetry factor calculation:

$$A_s = \frac{b}{a}$$

a = ascending part of the peak width at 10% of peak height

b = descending part of the peak width at 10% of peak height

The figure below shows a UV trace for acetone in a typical test chromatogram from which the HETP and A_s values are calculated.

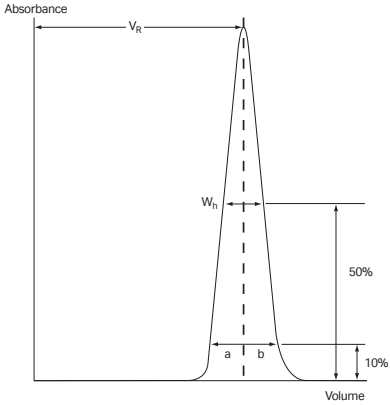

Fig 5. A typical test chromatogram showing the parameters used for HETP and A_S calculations.

7 Maintenance

For best performance from Q Sepharose High Performance and to maximize resin lifespan, follow the procedures below.

Equilibration

After packing and CIP, and before a chromatographic run, equilibrate with start buffer by washing with at least five bed volumes or until the column effluent shows stable conductivity and pH values. The equilibration step can be shortened by first washing with a high concentration buffer to obtain approximately the target pH value and then washing with start buffer until the conductivity and pH values are stable.

Regeneration

After each separation, elute any reversibly bound material with a high ionic strength solution (e.g., 1 to 2 M NaCl in elution buffer). Regenerate the resin by washing with five bed volumes of start buffer or until the column effluent shows stable conductivity and pH values.

CIP

Regular CIP prevents the buildup of impurities or contaminants in the packed bed and helps to maintain capacity, flow properties, and general performance. A specific CIP protocol must be designed for each process according to the type of impurities or contaminants present.

It is recommended to perform a CIP:

- before first-time use – especially after packing the column – or after long-term storage
- between cycles
- when an increase in back pressure or a reduction in column performance is observed
- to prevent potential cross-contamination or carry-over, when the same column is used for purification of different proteins or protein lots and batches

Recommended protocols for removing specific contaminants or impurities are described below.

Precipitated, hydrophobically bound proteins or lipoproteins	Wash with at least 3 CV of 1.0 M NaOH solution with reversed flow direction. Contact time depends on the type of contaminant present.
Ionically bound proteins	Wash with 0.5 to 2 CV of 2 M NaCl with reversed flow direction. Contact time 1 to 2 h.
Lipids and very hydrophobic proteins	Wash with 2 to 4 CV of 0.5% nonionic detergent (e.g., 1 M acetic acid) with reversed flow direction. Contact time 1 to 2 h. Alternatively, wash with 2 to 4 CV of up to 70% ethanol[1] or 30% isopropanol with reversed flow direction. Contact time 1 to 2 h.

[1] Consult local safety regulations to verify if safety precautions are required for the use of 70% ethanol or 30% isopropanol.

Sanitization

To reduce microbial contamination in the packed column, sanitization using 0.5 to 1.0 M NaOH with a contact time of 1 h is recommended. The CIP protocol for precipitated, hydrophobically bound proteins or lipoproteins sanitizes the resin effectively. For more information about the use of NaOH for sanitization, refer to *Application note (CY13951)*.

Storage

Store in 20% ethanol at a temperature of 4°C to 30°C.

After storage, equilibrate with at least five CV of start buffer.

8 Ordering information

Product	Pack size	Product code
Q Sepharose High Performance	75 mL	17101401
	1 L	17101403
	5 L	17101404
	10 L	17101405

Q Sepharose High Performance is supplied in suspension in 20% ethanol. For additional information, contact your local Cytiva representative.

Related products

Product	Pack size	Product code
PreDictor RoboColumn Q Sepharose HP	8 × 200 µL	28986103
PreDictor RoboColumn Q Sepharose HP	8 × 600 µL	28986192
HiTrap Q HP	5 × 1 mL	17115301
HiTrap Q HP	5 × 5 mL	17115401
HiScreen Q HP	1 × 4.7 mL	28950511
HiPrep Q HP 16/10	1 × 20 mL	29018182
Tricorn 5/100 column	1	28406410
Tricorn 10/100 column	1	28406415
HiScale 10/40	1	29360550
HiScale 16/20	1	28964441
HiScale 16/40	1	28964424
HiScale 26/20	1	28964514
HiScale 26/40	1	28964513
HiScale 50/20	1	28964445
HiScale 50/40	1	28964444

Accessories

Product	Pack size	Product code
Tricorn Glass Tube 5/100	1	18115306
Tricorn Packing Connector 5-5	1	18115321
Tricorn Packing Equipment 10/100	1	18115325
Packing tube 20, HiScale 10	1	29360551
Packing tube 20, HiScale 16	1	28986816
Packing tube 20, HiScale 26	1	28980383
Packing tube 20, HiScale 50	1	28980251
Packing tube 40, HiScale 16	1	28986815
Packing tube 40, HiScale 26	1	28964505
Packing tube 40, HiScale 50	1	28964506

Literature

Product	Reference
Application note	
Column efficiency testing	CY13149
Data file	
Q Sepharose High Performance, SP Sepharose High Performance	CY13443
Handbook	
Ion Exchange Chromatography: Principles and Methods	CY13983
Instructions	
Tricorn Empty High Performance Columns	28409488
HiScale columns (10, 16, 26, 50) and accessories	28967470

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