



Q Sepharose™ XL SP Sepharose™ XL

Ion exchange chromatography resins

Instructions for Use

Q and SP Sepharose™ XL are ion exchange chromatography resins designed to reduce the cost of manufacturing biopharmaceuticals by increasing the throughput of production-scale chromatography processes.

The high binding capacity of the resins, combined with high throughput, increases the productivity of manufacturing operations.

Main characteristics of Q and SP Sepharose XL include:

- capture of biomolecules directly from clarified samples for effective initial purification
- high dynamic binding capacity
- straightforward scale-up to production
- BioProcess™ resins for use in industrial downstream processing

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Read these instructions carefully before using the products.

Safety

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

1 Introduction

BioProcess chromatography 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 in process validation and submissions to regulatory authorities.

BioProcess resins cover all purification steps from the capture to the polishing step.

2 Resin properties

General

A chromatographic process for purifying a biomolecule can be divided into three steps:

- capture
- intermediate purification
- polishing

The goal of the capture step is to initially concentrate and purify the target molecule from a crude or clarified sample. Ion exchange is one of the techniques that is best suited for the capture step. As the sample might be viscous and contain proteases, a high loading volume and throughput are needed to give a rapid processing prior to the intermediate purification step.

Main properties

Sepharose XL is based on a strong, cross-linked, bead-formed agarose matrix. Dextran chains are coupled to the agarose matrix. Strong Q or SP ion exchange groups are attached to the dextran through chemically stable ether bonds.

Table 1. Main properties of Q Sepharose XL

Matrix	Cross-linked agarose, with dextran surface extender, spherical
Type of ion exchanger	Strong anion
Ionic capacity	0.18 to 0.26 mmol Cl ⁻ /mL resin
Particle size, d_{50v}¹	~ 90 µm
Chemical stability²	Stable in commonly used aqueous buffers, 1.0 M NaOH, 20% ethanol, 6 M guanidine hydrochloride
Physical stability	Negligible volume variation due to changes in pH or ionic strength
pH stability, operational³	2 to 12
pH stability, CIP⁴	2 to 14
pH ligand fully charged⁵	Entire pH range
Recommended operating flow velocity⁶	200 cm/h
Dynamic binding capacity, QB_{10%}⁷	≥ 160 mg BSA/mL resin
Temperature stability	4°C to 30°C
Avoid	Oxidizing agents
Storage	20% ethanol, 4°C to 30°C

¹ Median particle size of the cumulative volume distribution.

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

³ pH range where resin can be operated without significant change in function.

⁴ pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

⁵ 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.

⁶ 18 cm diameter, 20 cm bed height, at room temperature using buffers with the same viscosity as water.

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.

Table 2. Main properties of SP Sepharose XL

Matrix	Cross-linked agarose, with dextran surface extender, spherical
Type of ion exchanger	Strong cation
Ionic capacity	0.18 to 0.25 mmol H ⁺ /mL resin
Particle size, d_{50v}¹	~ 90 µm
Chemical stability²	Stable in commonly used aqueous buffers, 1.0 M NaOH, 20% ethanol, 6 M guanidine hydrochloride
Physical stability	Negligible volume variation due to changes in pH or ionic strength
pH stability, operational³	4 to 13
pH stability, CIP⁴	3 to 14
pH ligand fully charged⁵	Entire pH range
Recommended operating flow velocity⁶	200 cm/h
Dynamic binding capacity, QB_{10%}⁷	≥ 160 mg lysozyme/mL resin
Temperature stability	4°C to 30°C
Avoid	Oxidizing agents, long exposure to pH <4
Storage	0.2 M sodium acetate in 20% ethanol, 4°C to 30°C

¹ Median particle size of the cumulative volume distribution.

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

³ pH range where resin can be operated without significant change in function.

⁴ pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

⁵ 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.

⁶ 18 cm diameter, 20 cm bed height, at room temperature using buffers with the same viscosity as water.

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 Lysozyme in 50 mM Glycine-NaOH, pH 9.

3 Packing laboratory-scale columns

Recommended columns

Table 3. Laboratory-scale columns

Column	Compression factor ¹	Slurry concentration (%)	Bed height (mm)	Flow velocity (cm/h)	
				Step 1	Step 2
XK 16/40	1.15	50–70	10–30	100	600
XK 26/40	1.15	50–70	10–30	95	570
XK 50/30	1.15	50–70	10–28	90	540
HiScale™ 16/40	1.15	50–70	10–30	100	600
HiScale 26/40	1.15	50–70	10–30	95	570
HiScale 50/40	1.15	50–70	10–30	90	540

¹ Defined as the settled bed height (bed height measured after settling by gravity (L_{settled}) divided by the packed bed height (L_{packed}), i.e., $CF = L_{\text{settled}} / L_{\text{packed}}$.

Note: For lower bed heights, use columns with a 20 cm tube height instead of 40 cm.

Packing procedure

Step	Action
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|---|---|
| 1 | Assemble the column and, if necessary, the packing reservoir. |
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Step	Action
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|---|---|
| 2 | Remove any air from the column dead spaces by flushing the end piece and the adapter with packing buffer. |
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Note:

Make sure that no air is trapped below the column net.

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|---|---|
| 3 | Close the column outlet, leaving the net covered with packing buffer. |
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| 4 | Shake the resin container to resuspend the resin and let the bed sediment. Avoid stirring the sedimented resin. |
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|---|--|
| 5 | Mix the packing buffer with the resin to form a 50% to 70% slurry. |
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|---|--|
| 6 | Hold a glass rod against the column wall and pour the slurry along the glass rod in one continuous motion to minimize the introduction of air bubbles. |
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|---|---|
| 7 | If using a packing reservoir, immediately fill the remainder of the column and the reservoir with packing buffer. |
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| 8 | Connect the adapter or lid of the packing reservoir and connect the column to a pump. |
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Note:

Avoid trapping air bubbles below the adapter or in the inlet tubing.

Step	Action
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|----|--|
| 9 | Open the bottom outlet of the column and set the pump to run at the first target flow velocity (Step 1 value stated in the table above). |
| 10 | When the bed has stabilized, stop the pump and close the bottom outlet. |
| 11 | If using a packing reservoir, disconnect the reservoir and fit the adapter to the column. |
| 12 | With the adapter inlet disconnected, push the adapter approximately 2 mm down into the bed, allowing the packing solution to flush the adapter inlet. Lock the adapter in position. |
| 13 | Connect the pump, open the bottom outlet and continue packing for 15 minutes with the pump set to run at the second target flow velocity (Step 2 value stated in the table above). |
| | <i>Result:</i>
The bed compresses further and a space forms between the bed surface and the adapter. |
| 14 | Close the bottom outlet, disconnect the column inlet, and lower the adapter approximately 2 mm into the bed. |

The column is now packed.

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

4 Packing process-scale columns

Table 4. Recommended process-scale columns

Column	Description
BPG 100, 200, or 300 columns	Inner diameters 100 mm to 300 mm: for bed volumes up to 45 L at a maximum bed height of 30 cm. BPG columns are fitted with one adapter.
INdEX variable bed columns	Inner diameters from 70 mm to 200 mm; bed volumes up to 25 L at a maximum bed height of 61 cm.
Chromaflow™ variable and fixed bed columns	Inner diameters 400 mm to 2000 mm.

General packing recommendations

Columns can be packed in different ways depending on the type of column and equipment used. Always read and follow the relevant column instructions carefully.

Q and SP Sepharose XL are straightforward to pack since their rigidity allows for the use of high flow rates.

The different packing methods that are suitable for Sepharose XL ion exchangers are:

- pressure packing for columns with adapters, for example BPG columns.
- hydraulic pressure packing, for example, INdEX columns.
- Chromaflow packing method for standard Chromaflow columns

The quality of the column packing greatly affects the separation result. It is important to pack and test the column according to the recommendations.

Determine the optimal packing flow rate before performing the packing procedure.

Determine the optimal packing flow rate

The optimal packing flow rate is dependent on column size and type, bed height, packing solution, and temperature. Determine the optimal packing flow rate for each individual system. Follow the detailed instructions in the column *User Manual* which is supplied with your column.

The following guidelines are for fixed bed height columns and columns with adapters.

Step	Action
1	Calculate the exact amount of resin needed for the slurry. The amount of resin required per liter packed bed is approximately 1.15 L sedimented resin. Note: <i>Calculating the exact amount of resin is especially important for columns with fixed bed heights.</i>
2	Prepare the column exactly as for column packing.
3	Start packing the column at a low flow rate (for example 30% of the expected maximum flow rate) and record the flow rate and back pressure when the bed is packed and the pressure has stabilized.
4	Increase the flow rate in small steps and record the flow rate and pressure at each step after the pressure has stabilized.

Step	Action
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5	Continue recording the flow rate and the pressure until the maximum flow rate is reached, that is, when the flow rate levels off at a plateau (indicating bed compression), or when the pressure reaches the pressure specification of the column used.
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6	Plot the pressure against the flow rate. The optimal packing flow rate/pressure is 70% to 100% of the maximum flow rate/pressure.
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Note:

The operational flow rate/pressure must be < 70% of the packing flow rate/pressure.

Packing the column

Follow the detailed instructions in the user manual supplied with the column.

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

5 Evaluation of the packed column

Introduction

The packing quality needs to be checked by column efficiency testing. Testing must be performed after packing and at regular intervals during the working life of the column and also when the separation performance is seen to deteriorate.

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 NaCl 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.

Changing the solute, solvent, eluent, sample volume, or flow velocity, influences the results.

Method for measuring HETP and A_s

Method parameters:

Parameter	Description
Sample volume	2.5% of the bed volume
Sample concentration	1.0% (v/v) acetone
Flow velocity	20–30 cm/h
UV	280 nm, 1 cm, 0.1 AU

To avoid dilution of the sample, apply it as close to the column inlet as possible and calculate HETP and A_s from the UV curve (or conductivity curve) as follows:

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

L = bed height (cm)

N = number of theoretical plates

N is calculated as follows:

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

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

To facilitate comparison of column performance, the concept of reduced plate height is often used. Calculate the reduced plate height as follows:

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

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

d = diameter of the particle

As a guideline, a value of < 3 is very good.

The peak should be symmetrical with an asymmetry factor as close to 1 as possible. A change in the shape of the peak is usually the first indication of bed deterioration due to excessive use. A typical acceptable range is $0.8 < A_s < 1.8$.

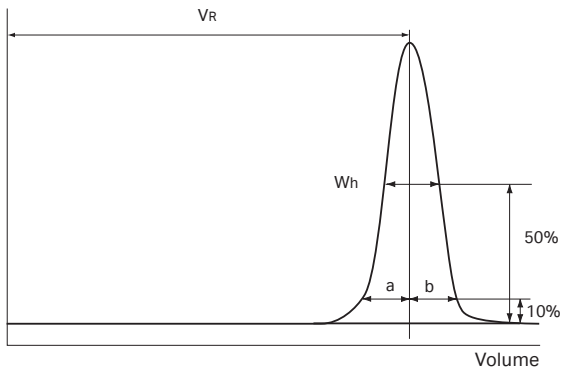
Calculate the peak asymmetry as follows:

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

a = 1st half peak width at 10% of peak height.

b = 2nd half 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.



Column: BPG 300

Flow velocity: 19 cm/h

Resin: Sepharose 6 Fast Flow

W_h : 0.9

Bed height: 57.5 cm

HETP: 0.024 cm

Bed volume: 40.6 L

a: 0.90

Sample: 1.05 L (1% acetone)

b: 0.85

Eluent: Ultrapure water

A_S : 0.94

6 Method design and optimization

Method design

The primary aim of method design is to establish conditions that enable binding the highest amount of target molecule, in the shortest time and with the highest product recovery.

The very large loading capacities available throughout the structures of Q and SP Sepharose XL mean that the resins can bind very large amounts of the target molecules. For maximum capacity, it might be necessary to add a low NaCl concentration (typically, 10 to 50 mM) to the sample, especially for SP Sepharose XL. Special attention must therefore be given to optimize the elution conditions to avoid tailing when eluting the target protein. Fully optimized elution also increases the effectiveness of CIP procedures, which makes the whole purification process more efficient.

Throughput optimization

When optimizing for highest throughput and productivity, consider the following:

- Sample load.
- Sample application time.
- Acceptable loss in product recovery.
- For maximum capacity, it might be necessary to add a low NaCl concentration (typically, 10 to 50 mM) to the sample, especially for SP Sepharose XL.

Determine the dynamic binding capacity for the target protein by frontal analysis using a real process sample. Since the dynamic binding capacity is a function of the flow rate/residence time applied during sample application, the breakthrough capacity must be defined over a range of different flow rates to reveal the optimum level that gives the highest throughput without excessive product leakage at the column outlet.

7 Scale-up

After optimizing the method at laboratory scale, the process can be scaled up. Scale-up to larger columns is typically performed by keeping the bed height and flow velocity (cm/h) constant while increasing the bed diameter and the flow rate (mL/h).

Scale-up procedure

Step	Action
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| 1 | Choose a bed volume according to the required binding capacity. |
| 2 | Choose a column diameter to obtain a bed height of 5 to 15 cm so that high flow rates can be used. |

Note:

Maximum flow rate decreases with bed height.

Operate at no more than 70% of the maximum flow velocity.

- | | |
|---|--|
| 3 | Define flow velocity during sample application to check that the residence time is not shorter than the established time in the small-scale experiments. The residence time is calculated as the bed volume (mL) divided by the volumetric flow rate (mL/min) applied during sample loading. |
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Step	Action
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|---|---|
| 4 | Check the buffer delivery and monitoring systems for time delays or volume changes. |
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Note:

The use of larger systems when scaling up might cause deviations from the optimized method at the small-scale. For example, outlet tubing of different lengths and diameter can cause zone spreading on larger systems.

- | | |
|---|--|
| 5 | Keep sample concentration and gradient slope constant. |
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8 Cleaning-In-Place

Introduction

Cleaning-In-Place (CIP) is a procedure that removes impurities or contaminants from the resin and the purification system. Accumulation of impurities or contaminants might affect the chromatographic properties of the column, for example, blocking the column, increasing the back pressure, and reducing the flow rate. Regular CIP prevents the buildup of the impurities or contaminants in the resin bed, and helps to maintain the capacity, flow properties, and general performance of the resins.

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 cross-over, when the same column is used for purification of different proteins or protein lots and batches

CIP procedure

For removal of precipitated or denatured substances, follow the procedure below:

Step	Action
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|---|---|
| 1 | Wash with two column volumes of 6 M guanidine HCl. |
| 2 | Wash immediately with at least 5 column volumes of binding buffer, pH 7 to 8. |

Note:

Use reversed flow direction.

For removal of hydrophobically bound substances, CIP is performed as follows:

Step	Action
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|---|--|
| 1 | Wash the column with two column volumes of a bound substances nonionic detergent (e.g., conc. 0.1%). |
| 2 | Wash immediately with at least five column volumes of binding buffer, pH 7 to 8. |

Note:

Use reversed flow direction.

The following is another CIP protocol for removal of hydrophobically bound substances:

Step	Action
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|---|---|
| 1 | Wash the column with three to four column volumes of 70% ethanol. |
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Note:

Use reversed flow direction and an increasing gradient to avoid air bubble formation when using high concentrations of organic solvents.

Note:

Consult local safety regulations to verify if safety precautions are required for the use of 70% ethanol.

- | | |
|---|--|
| 2 | Wash immediately with at least five column volumes of binding buffer, pH 7 to 8. |
|---|--|

9 Sanitization

Sanitization reduces microbial contamination of the bed to a minimum. Follow the steps below to sanitize the column:

Step	Action
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|---|---|
| 1 | Equilibrate the column with 20% ethanol. |
| 2 | Allow to stand for 12 hours. |
| 3 | Wash with at least five column volumes of binding buffer. |

For more details, refer to Application note *Use of sodium hydroxide for cleaning and sanitization of chromatography resins and systems (CY13951)*.

10 Storage

Store unused resin in its container at 4°C to 30°C and make sure that the screw top is fully tightened.

Store packed resin by equilibrating the packed column with a buffer containing 20% ethanol (Q Sepharose XL) or 20% ethanol and 0.2 M sodium acetate (SP Sepharose XL) to prevent microbial growth.

Note: *Before use, equilibrate with at least five bed volumes of the starting buffer.*

11 Ordering information

Q Sepharose XL is supplied as a suspension in 20% ethanol. SP Sepharose XL is supplied as a suspension in 20% ethanol and 0.2 M sodium acetate. For additional information, including Data files, application references and Regulatory Support File, contact your local Cytiva representative.

Product	Pack size	Product code
Q Sepharose XL	300 mL	17507201
	5 L	17507204
	10 L ¹	17507205
	60 L ¹	17507260
SP Sepharose XL	300 mL	17507301
	5 L	17507304
	10 L	17507305
	60 L ¹	17507360

¹ Pack size available upon request.

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