cytiva

Q **Sepharose** XL
SP **Sepharose** XL

Ion exchange chromatography

Instructions for Use

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

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

Key characteristics of Q and SP Sepharose XL include:

- Capture of biomolecules directly from the clarified feed-stocks for effective initial purification
- Dynamic binding capacities up to 10 fold higher than conventional ion exchangers
- Easy production scale up
- Belong to 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. 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.

2 Characteristics

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 feed-stock. Ion exchange is one of the techniques that is best suited for the capture step. As the feedstock may be viscous and contain proteases, high loading volume and throughput are needed to give a rapid processing prior to the Intermediate Purification step.

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

Properties

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

Table 1. Characteristics of Q Sepharose XL

Matrix	Cross-linked agarose, with dextran surface extender, spherical
Type of ion exchanger	Strong anion
Ionic capacity	0.18-0.26 mmol Cl^-/mL resin
Particle size, d50V[1]	~ 90 µm
Chemical stability	Stable to commonly used aqueous buffers, 1.0 M NaOH[2], 20% ethanol, 6 M guanidine hydrochloride
Physical stability	Negligible volume variation due to changes in pH or ionic strength
pH stability, operational[3]	2 to 12
pH stability, CIP[4]	2 to 14
pH ligand fully charged[5]	Entire pH range
Recommended operating flow velocity	200 cm/h[6]
Dynamic binding capacity, QB10[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. Characteristics of SP Sepharose XL

Matrix	Cross-linked agarose, with dextran surface extender, spherical
Type of ion exchanger	Strong cation
Ionic capacity	0.18-0.25 mmol H^+/mL resin
Particle size, d50V[1]	~ 90 µm
Chemical stability	Stable to commonly used aqueous buffers, 1.0 M NaOH[2], 20% ethanol, 6 M guanidine hydrochloride
Physical stability	Negligible volume variation due to changes in pH or ionic strength
pH stability, operational[3]	4 to 13
pH stability, CIP[4]	3 to 14
pH ligand fully charged[5]	Entire pH range
Recommended operating flow velocity	200 cm/h[6]
Dynamic binding capacity, QB10[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 lab-scale columns

Recommended columns

Table 3. Lab-scale columns

Name	Description
XK 16/20	i.d. 16 mm, bed volumes up to 30 mL at bed height up to 15 cm.
XK 26/20	i.d. 26 mm, bed volumes up to 80 mL at bed height up to 15 cm.

Packing procedure

Step	Action
1	Assemble the column and packing reservoir, if necessary.
2	Remove the air from the column dead spaces by flushing the end-piece and adapter with packing buffer. ***Note:*** *Check that no air is trapped under the column net.*
3	Close the column outlet leaving the net covered with packing buffer.
4	Decant the resin storage solution and replace it with the packing buffer.
5	Resuspend the resin stored in its container by shaking and let the bed sediment. Avoid stirring the sedimented resin.

Step	Action
6	Mix the packing buffer with the resin to form a 50% to 70% slurry. ***Note:*** *Sedimented bed volume/slurry volume = 0.5 to 0.7*
7	Pour the slurry through a small funnel into the column. Hold the funnel end near the column wall to make the slurry flow along the wall. This prevents air bubbles from occurring.
8	If using a packing reservoir, immediately fill the remainder of the column and reservoir with packing buffer.
9	Connect the adapter or lid of the packing reservoir and connect the column to a pump. ***Note:*** *Avoid trapping air bubbles under the adapter or in the inlet tubing.*
10	Open the bottom outlet of the column and set the pump to run at the desired flow velocity. ***Note:*** *Ideally, Sepharose XL resins are packed at an initial flow velocity of 250 cm/h until the resin has settled.*
11	When the bed has stabilized, close the bottom outlet and stop the pump.
12	If using a packing reservoir, disconnect the reservoir and fit the adapter to the column.

Step	Action
13	With the adapter inlet disconnected, push the adapter down, approximately 2 mm into the bed, allowing the packing solution to flush the adapter inlet.
14	Connect the pump, open the bottom outlet and continue packing at 600 cm/h for 15 minutes. *Result:* The bed compresses further and a space froms between the bed surface and the adaptor.
15	Close the bottom outlet and disconnect the column inlet and lower the adapter approximately 2 mm into the bed. *Result:* The column is now packed.

4 Packing process-scale columns

Table 4. Recommented Process-scale columns

Name	Description
BPG 100, 200 or 300 columns	Inner diameters 100 mm to 300 mm: for bed volumes up to 45 liters at a maximum bed height of 30 cm. BPG columns are fitted with one adaptor.
INdEX variable bed columns	Inner diameters from 70 to 200 mm; bed volumes up to 25 liters at a maximum bed heights of 61 cm.
Chromaflow™ variable and fixed bed columns	Inner diameters 280–2000 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 instruction manual carefully.

Q and SP Sepharose XL are easy to pack since their rigidity allows the use of high flow rates. Columns can be packed in different ways depending on the type of column and equipment used. Always read and follow the relevant column instruction manual carefully.

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

- Pressure packing for columns with adaptors, for example BPG columns.
- Hydraulic pressure packing, for example, INdEX columns.
- Chromaflow packing method for standard Chromaflow columns

How well the column is packed has a major effect on the separation result. It is important to pack and test the column according to the recommendations.

Start the packing procedure by determining the optimal packing flow rate.

Determining optimal packing flow rate

The optimal packing flow velocity is dependent on column size and type, bed height, packing solution and temperature. The optimal packing flow rate must, be determined empirically for each individual system. Follow the detailed instructions in the Column *User Manual* which is supplied with your column. Copies can be ordered from your local representative

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

Step	Action
1	Calculate the exact amount of the resin needed for the slurry. The quantity of resin required per liter packed bed is approximately 1.15 liters sedimented resin. ***Note:*** *Calculating exact amount of the resin is especially important for columns with fixed bed heights.*
2	Prepare the column exactly as for column packing.
3	Start packing the column at a low flow velocity (e.g., 30% of the expected max flow velocity) and record the flow velocity and back pressure when the bed is packed and the pressure has stabilized.
4	Increase the flow velocity in small steps and record the flow velocity and pressure at each step after the pressure has stabilized.
5	Continue recording flow and pressure until the maximum flow velocity is reached, that is when the flow velocity levels off at a plateau (indicating bed compression), or when the pressure reaches the pressure specification of the column used.

Step	Action
6	Plot pressure against flow rate as indicated in *Figure 1, on page 13*. The optimal packing flow rate /pressure is 70% to 100% of the maximum flow rate/pressure. ***Note:*** *The operational flow rate/pressure should be < 70% of the packing flow rate/pressure.*

5 Packing evaluation

Introduction

The packing quality needs to be checked by column efficiency testing. The test must be done after the 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 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 is depended 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, 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	15 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:

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

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:

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

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 $0.8 < A_s < 1.8$.

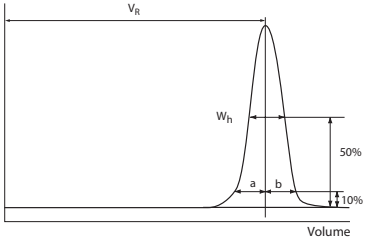
Calculate the peak asymmetry as follows:

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

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

b = 2nd half peak width at 10% of peak height.

Figure 1, on page 13 shows a UV trace for acetone in a typical test chromatogram from which the HETP and A_s values are calculated.



Column: BPG 300
Resin: Sepharose 6 Fast Flow
Bed height: 57.5 cm
Bed volume: 40.6 L
Sample: 1.05 L (1% acetone)
Eluent: Ultra pure water
Flow rate: 19 cm/h
$W_h = 0.9$
HETP = 0.024 cm
a: 0.90
b: 0.85
A_s: 0.94

Fig 1. UV trace for acetone in a typical test chromatogram showing the HETP and A_s calculations.

6 Method design and optimization

Method design

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

The very high loading capacities available throughout the structures of Q and SP Sepharose XL mean that they can bind exceptionally large amounts of target material. Special attention must therefore be given to optimizing elution conditions to avoid tailing when the eluting the protein of interest. Fully optimized elution also increases the effectiveness of CIP procedures, which will make the whole purification process more efficient.

Throughput optimization

When optimizing for highest throughput and productivity, it is necessary to define the highest sample load in the shortest sample application time with the most acceptable loss in the product recovery.

The dynamic binding capacity for the target protein should be determined by frontal analysis using real process feed-stock. Since the dynamic binding capacity is a function of the flow velocity applied during sample application, the breakthrough capacity must be defined over a range of different flow velocities to reveal the optimum level that gives highest throughput without excessive leakage of product at the column outlet.

7 Scaling 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.

Scale-up procedure

Step	Action
1	Choose the bed volume according to the required binding capacity.
2	Choose the column diameter to obtain the bed height of 5 to 15 cm so that high flow rates can be used. ***Note:*** *Maximum flow rate is inversely proportional to the 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 equal to the bed height (cm) divided by the flow velocity (cm/h) applied during sample loading.

Step	Action
4	Check the buffer delivery and monitoring systems for time delays or volume changes. ***Note:*** *The use of larger systems when scaling up, may cause some deviations from the optimized method at the small-scale. For example different lengths and diameters of outlet tubing can cause zone spreading on larger systems.*
5	Keep sample concentration and gradient slope constant.

8 Cleaning-In-Place

Cleaning-In-Place (CIP) is used for removal of very tightly bound, precipitated or denatured substances from the resin and the purification system. Accumulation of contaminants may 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 build up of the contaminants in the packed bed, and helps to maintain the capacity, flow properties and general performance of the resins.

The following CIP protocols should be used as guidelines. Depending on the nature of the contaminants, different protocols need to be used and if the fouling is severe, the protocols may need further optimization. The CIP frequency depends on the nature of the feed material. The recommendation is to use a CIP procedure at least every five cycles of normal use.

CIP procedure

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

Step	Action
1	Wash with two column volumes of 6 M guanidine hydrochloride.
2	Wash immediately with at least 5 column volumes of filter-sterilized binding buffer, pH 7 to 8. ***Note:*** *Use reversed flow direction.*

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

Step	Action
1	Wash the column with two column volumes of a bound substances nonionic detergent (e.g., conc. 0.1%).

Step	Action
2	Wash immediately with at least five column volumes of filter-sterilized 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
1	Wash the column with three to four column volumes of 70% ethanol. ***Note:*** *Use reversed flow direction and an increasing gradient to avoid air bubble formation when using high concentrations of organic solvents.*  **CAUTION** 70% ethanol can require the use of explosion-proof areas and equipment.
2	Wash immediately with at least five column volumes of filter-sterilized 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
1	Equilibrate the column with 20% ethanol. Equilibrate the column with a solution of 20% ethanol.
2	Allow to stand for 12 hours.
3	Wash with at least five column volumes of sterile binding buffer.

10 Storage

Store the unused or packed resins as follows:

When	Then
Unused resin	In its container at 4°C to 30°C, and check that the screw top is fully tightened.
Packed column	Equilibrate the packed column in a buffer containing 20% ethanol 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	Quantity	Product code
Q Sepharose XL	300 mL	17507201
	5 L	17507204
	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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