GE Healthcare
Life Sciences

Q Sepharose™ XL
SP Sepharose XL

Q Sepharose XL and SP Sepharose XL ion exchange chromatography media (resins) are specially designed for use in packed beds to capture biomolecules from clarified feed-stocks. The high loading capacities of the media, combined with high throughput, can increase the productivity of manufacturing operations. In addition, Q and SP Sepharose XL belong to the BioProcess™ media family and are fully documented and supported to meet the demands of downstream processing including regulatory support files, recommended columns, and packing procedures.

- Capture biomolecules directly from clarified feed-stocks for effective initial purification
- High dynamic binding capacity
- Easy scale-up to production
- Belong to BioProcess media family for perfomance and support to match their use in industrial downstream processing

Fig 1. Q Sepharose XL and SP Sepharose XL media combine high binding capacity with good resolution.

Ion exchange chromatography

Ion exchange chromatography is probably the most frequently used and versatile method for fractionating proteins and peptides, even those with small differences in charge. Furthermore, binding and elution conditions are easy to optimize, resulting in fast separations that are reproducible and cost-effective to scale up.

The technique is based on reversible interactions between charged molecules and immobilized ion exchange groups of opposite charge. The charged molecules bind to the separation medium at low ionic strength and are then eluted with a salt or pH gradient. Continuous gradient elution is most often used when good resolution is needed, while simple stepwise gradient elution is employed for sample preparation, group separation, or concentration.

Chromatography media characteristics

Long chains of dextran are coupled to a highly cross-linked agarose matrix.

The stable agarose matrix and long, flexible chains of dextran with bound charged groups work together to increase loading capacity whilst allowing high flow rates.

The dextran greatly increases the exposure of target molecules to the Q or SP charged groups. The long dextran molecules are flexible enough to allow passage of charged protein molecules. The overall result is increased loading capacity (up to 10-fold higher than conventional Sepharose Fast Flow ion exchangers). With their high loading capacities and excellent physical and chemical stability, they are well-suited to use in the capture step of a downstream process that is characterized by large loads of crude samples.

Q Sepharose XL has been shown to have excellent selectivity and capacity for viruses and in particular adenoviruses, an important vector for gene therapy.

Q Sepharose XL and SP Sepharose XL chromatography media use only biocompatible materials well-documented in commercial production.

Sepharose XL is based on a highly cross-linked, bead-formed 6% agarose matrix similar to the well established Q and SP Sepharose Fast Flow media. Dextran chains are covalently coupled to the agarose matrix. Strong Q and SP ion exchange groups are attached to the dextran through chemically stable ether bonds.

Tables 1 and 2 list the main characteristics of Q and SP Sepharose XL.

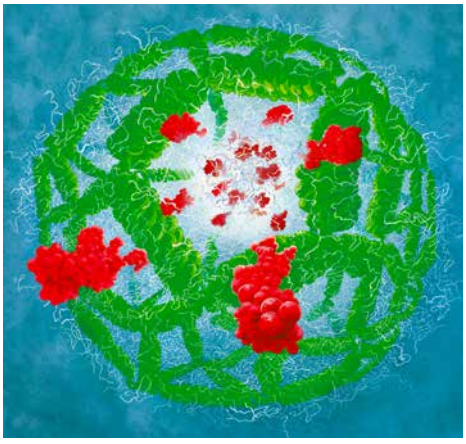


Fig 2. Shows an artist's impression of protein molecules binding to an XL ion exchanger.

Table 1. Characteristics of Q Sepharose XL

Type of ion exchanger	Strong anion
Ionic capacity	0.18 to 0.26 mmol Cl^-/mL medium
Matrix structure	Cross-linked 6% agarose with bound dextran
Particle form	Spherical, 45 to 165 µm
Mean particle size	90 µm
Chemical stability[1]	– Stable in all commonly used aqueous buffers – 1 M NaOH – 20% ethanol – 6 M guanidine-HCl Avoid: – Long exposure (1 w, 20 °C) to pH < 4 – Oxidizing agents – Anionic detergents
Recommended pH working cleaning-in-place	 2 to 12 2 to 12
Recommended working flow velocity	300 to 500 cm/h
Binding capacity[2]	> 130 mg bovine serum albumin/mL medium
Temperature stability	4 to 40°C
Storage	20% ethanol

[1] *No significant change in ionic binding capacity and carbon content after 1 w storage at 40°C.*

[2] *Breakthrough capacity at 10% of 2 mg bovine serum albumin/mL in a 4.4 mL packed bed at a linear velocity of 300 cm/h in 50 mM Tris-HCl, pH 7.5, bed height 10 cm.*

Table 2. Characteristics of SP Sepharose XL

Type of ion exchanger	Strong cation
Ionic capacity	0.18 to 0.25 mmol H^+/mL adsorbent
Matrix structure	Cross-linked 6% agarose with bound dextran
Particle form	Spherical, 45 to 165 µm
Mean particle size	90 µm
Chemical stability[1]	– Stable in all commonly used aqueous buffers – 1 M NaOH – 20% ethanol – 6 M guanidine-HCl Avoid: – Long exposure (1 w, 20°C) to pH < 4 – Oxidizing agents – Cationic detergents
Recommended pH working cleaning-in-place	 4 to 13 4 to 13
Recommended working flow velocity	300 to 500 cm/h
Binding capacity[2]	> 160 mg lysozyme/mL medium
Temperature stability	4 to 40°C
Storage	0.2 M sodium acetate in 20% ethanol

[1] *No significant change in ionic binding capacity or carbon content after 1 w storage at 40°C.*

[2] *Breakthrough capacity at 10% of 2 mg lysozyme/mL in a 4.4 mL packed bed at a linear velocity of 300 cm/h in 50 mM glycine-HCl, pH 9.0, bed height 10 cm.*

High loading capacity

Q Sepharsoe XL and SP Sepharose XL expose charged groups in an optimized fashion for binding of target molecules. Thus, the dynamic binding capacity of Q and SP Sepharose XL is very high.

Figures 3 and 4 show breakthrough curves for SP Sepharose XL at different flow velocities and bed heights. As expected, increased bed height gives steeper breakthrough curves and capacity, while increased flow velocity reduces capacity.

High resolution at high loading

The high binding capacities of Q Sepharose XL and SP Sepharose XL is combined with good resolution. Figure 4 shows that increasing sample load up to 10% of the breakthrough capacity has little effect on the resolution of the three peaks. The selectivities of Q and SP Sepharose XL are similar to the corresponding Sepharose Big Beads and Sepharose Fast Flow media. The similarity can speed up method development on Sepharose XL ion exchangers.

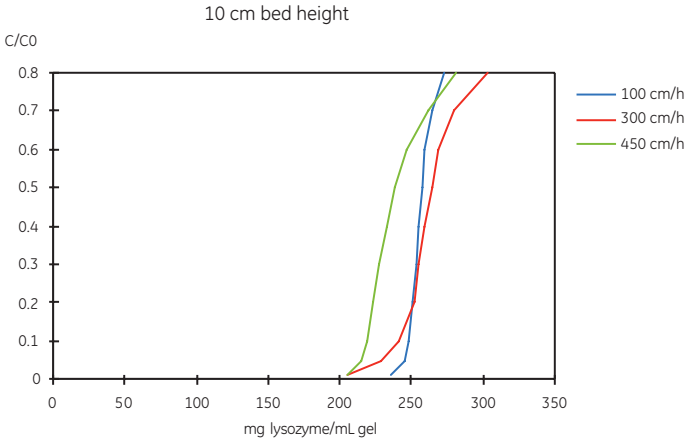


Fig 3. Dynamic binding capacity for lysozyme at different flow velocities on SP Sepharose XL packed in an XK 16/20 column at 10 cm bed height.

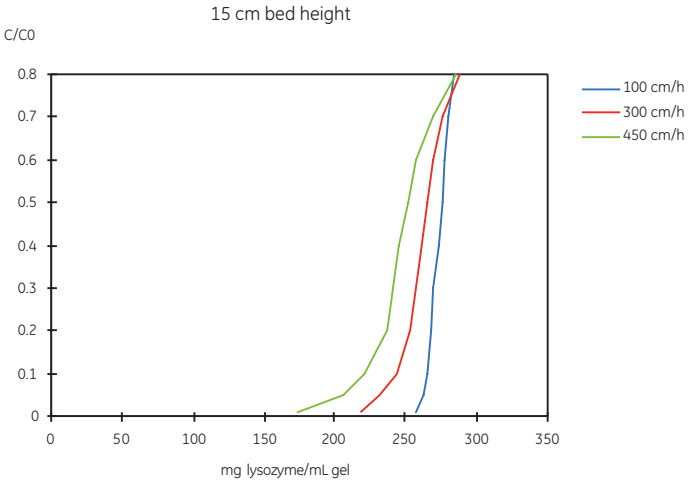


Fig 4. Dynamic binding capacity for lysozyme at different flow velocities on SP Sepharose XL packed in an XK 16/20 column at 15 cm bed height.

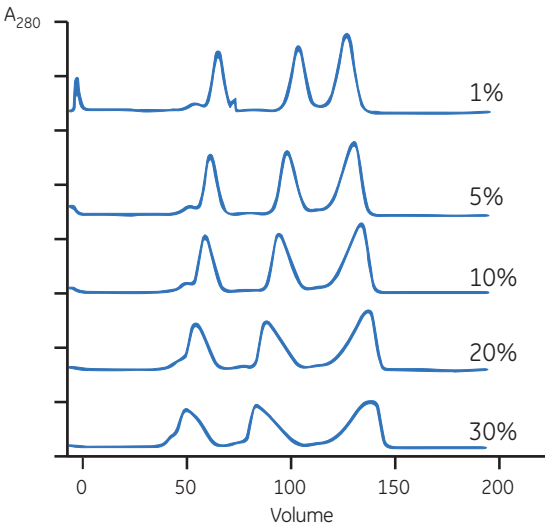


Fig 5. Separation of ribonuclease, cytochrome C, and lysozyme on SP Sepharose XL. Increasing sample loading up to 10% has little effect on resolution. At 30% the peaks are broader, but still with acceptable resolution.

High chemical and mechanical stabilities give flexibility and long working life

Q Sepharose XL and SP Sepharose XL have high chemical stability and can be used over a wide pH range. The flexibility offers considerable freedom when choosing adsorption and elution conditions, which helps in optimizing the separation. In addition, effective cleaning and sanitization procedures can be applied, which extend the working life of the media and increase the overall process economy.

Operation

Scale up

Start by optimizing the separation at laboratory scale, and then scale up the process by keeping the linear flow rate and sample-to-bed volume ratio constant, and increasing the column diameter. We recommend bed heights of 5 to 15 cm.

Equipment

Q Sepharose XL and SP Sepharose XL can be used with most equipment available for chromatography, from laboratory to production scale. Table 3 lists recommended columns available from GE Healthcare.

Table 3. Recommended columns for Q and SP Sepharose XL

Column	Inner diameter (mm)	Bed volume	Bed height (cm)
Lab-scale:			
HiScale™ 16/20	16	up to 40 mL	max. 20
HiScale 26/20	26	up to 80 mL	max. 20
Production scale:			
AxiChrom™	50 to 200	0.6 to 9.4 L	30
	300 to 1000	21 to 236 L	30
BPG 100/500	100	up to 2.4 L	max. 30
BPG 200/500	200	up to 9.4 L	max. 30
BPG 300/500	300	up to 21 L	max. 30
BPG 450/500	450	up to 43 L	max. 27
Chromaflow™ V400/100–300	400	37	30
Chromaflow V600/100–300	600	84	30
Chromaflow V800/100–300	800	150	30
Chromaflow[1] V1000/100–300	1 000	2236	30

[1] *Chromaflow columns are available even in larger diameters, please contact your GE Healthcare representative for information.*

Recommendations for packing, method design and optimization, and cleaning and sanitization of Q Sepharose XL and SP Sepharose XL are included in instructions supplied with each pack of medium.

Applications

Purification of recombinant α-amylase from E. coli

Recombinant α-amylase (M_r 50 000) was produced in *E. coli*. After homogenisation, a purification method was delevoped on Q Sepharose XL and scaled up to pilot level.

The first step of method development was to determine optimal pH. This was performed by method scouting in an XK 16/20 column run on ÄKTA™ chromatography system. Scouting between pH 6 and 9 revealed that pH 8 was the optimum.

Scouting on ÄKTAexplorer was also used to determine maximum loading. The homogenized starting material was diluted to a conductivity below 10 mS/cm. To increase binding capacity for the target protein, 10 mM $CaCl_2$ was added to precipitate DNA. Breakthrough curves were generated with increasing amounts of starting material until α-amylase was detected in the flowthrough.

Loading was reduced to 75% of the maximum capacity and verified before the method was scaled up to an INdEX 70 column.

The specific activities of α-amylase in the eluates from the XK 16/20 and INdEX 70 columns were 6180 and 6420 U/L respectively. Recoveries were approximately 60%. (Note that methods used to determine the protein content of crude feedstocks are known to give rather unreliable results, which may explain this low recovery.)

Capture: the initial purification step

A chromatographic process for purifying a biomolecule can be divided into three stages: capture, intermediate purification, and polishing. Each has its own goals. The goal of capture step is initial concentration and purification of the molecule of interest from crude or clarified feedstock. Ion exchange is a technique well suited for capture.

As the feedstock may contain high levels of impurities including proteases, high loading and throughput are needed for rapid processing and product stabilization prior to the intermediate purification step.

Figure 5 shows the chromatogram of the pilot scale separation. Table 4 shows the DNA content of different fractions from the separation. Note that most of the DNA elutes after α-amylase and that relatively little is found in the α-amylase peak. The result illustrates that the ion exchange separation on Q Sepharose XL reduces the amount of DNA in the fraction containing the target protein.

Figure 6 illustrates the purity of α-amylase captured at pilot scale.

Table 4. DNA content in fractions from purification of α-amylase on Q Sepharose XL in an INdEX 70 column

Fraction	ng DNA/µL
Starting material	44
Flowthrough	21.5
1st peak (containing α-amylase)	10
2nd peak	117

Column:	INdEX 70 (70 mm i.d.)
Adsorbent:	Q Sepharose XL, 385 mL bed volume
Sample:	Recombinant α-amylase produced in *E. coli*, homogenized, 2.2 L diluted in distilled water to 15.4 L, 7.2 mS/cm, 10 mM $CaCl_2$, centrifuged
Start buffer:	20 mM Tris-HCl, 10 mM $CaCl_2$, pH 8
Elution buffer:	20 mM Tris-HCl, 1 M NaCl, 10 mM $CaCl_2$, pH 8
Flow rate:	12 L/h (300 cm/h)
Gradient:	20 bed volumes 0 to 1 M NaCl
Eluate:	1.48 L, 3.8 bed volumes
Spec. act. α-amylase	6420 U/L

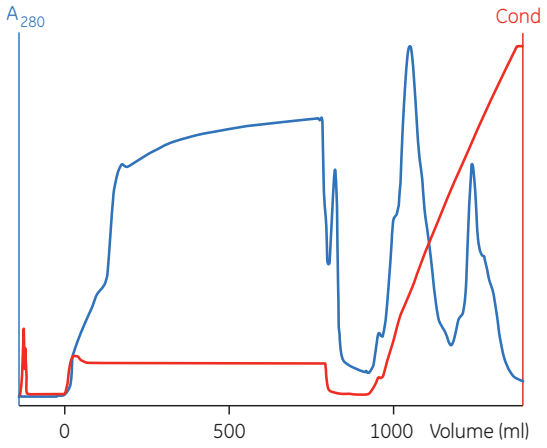
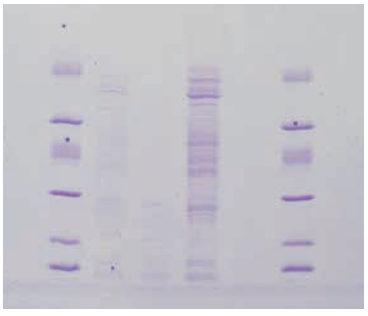


Fig 5. Pilot scale capture of recombinant α-amylase from *E. coli* on Q Sepharose XL.



Lanes
1. –
2. LMW markers
3. Starting material
4. Flowthrough
5. 1st peak (containing α-amylase)
6. 2nd peak
7. LMW markers

Fig 6. SDS-PAGE (reduced, Coomassie™ Brilliant Blue staining) of starting material and eluted fractions from the capture of *E. coli* recombinant α-amylase on Q Sepharose XL.

BioProcess media: media made for bioprocessing

BioProcess media are a specially selected range of chromatography media developed and supported for process-scale chromatography. All media are produced following validated methods and are tested for that they meet the performance requirements of the manufacturing industry. Regulatory support files contain information to assist process validation and submissions to regulatory authorities. BioProcess media cover all purification steps, from capture to polishing.

Ordering information

Product	Quantity	Code number
Q Sepharose XL	300 mL	17-5072-01
	5 L	17-5072-04
	10 L	17-5072-05
	60 L	17-5072-60
SP Sepharose XL	300 mL	17-5073-01
	5 L	17-5073-04
	10 L	17-5073-05
	60 L	17-5073-60

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.

Related products

HiTrap™ Q XL	5 × 1 mL	17-5158-01
	5 × 5 mL	17-5159-01
HiTrap SP XL	5 × 1 mL	17-5160-01
	5 × 5 mL	17-5161-01

Empty columns

*Column**	
HiScale 16/20	28-9644-41
HiScale 16/40	28-9644-24
HiScale 26/20	28-9645-14
HiScale 26/40	28-9645-13
HiScale 50/20	28-9644-45
HiScale 50/40	28-9644-44

* Each HiScale column is delivered with two adapters

Related literature

Data File

Axichrom columns	28-9290-41

Handbook

Ion Exchange Chromatography, Principles and Methods	18-1114-21

Selection guides

Ion exchange columns and media	18-1127-31
Prepacked chromatography columns for AKTA™ systems	28-9317-78

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18-1123-82 AF 06/2013