Q Sepharose XL
Q Sepharose XL virus licensed
SP Sepharose XL

Introduction

Q and SP Sepharose™ XL are ion exchange adsorbents specially designed for use in packed beds to capture biomolecules from clarified feed-stocks. Their extremely high loading capacities, combined with high throughput, can increase the productivity of manufacturing operations. In addition, Q and SP Sepharose XL are fully documented and supported to meet the demands of downstream processing. Q Sepharose XL virus licensed is specifically intended for the purification of viruses.

Important information

Separating viral particles with "Q Sepharose XL" products may require a license under United States Patent 6,537,793 B2 and foreign equivalents owned by Gencell SAS. Such a license is not included with the purchase of Q Sepharose XL but is included with the purchase of "Q Sepharose XL virus licensed" products.

With the purchase of "Q Sepharose XL virus licensed" the customer is granted a free limited license under United States Patent 6,537,793 B2 and foreign equivalents owned by Gencell SAS to separate viral particles solely through use of the product purchased.

XL media – the inside story

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 the Q or SP charged groups. The long dextran molecules are flexible enough to allow passage of charged protein molecules. The overall result is extreme loading capacity (up to 10 fold higher than conventional ion exchangers).

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

Fig 1. Shows an artist's impression of protein molecules binding to an XL ion exchanger.

- Capture biomolecules directly from clarified feed-stocks for effective initial purification
- Dynamic binding capacities up to 10 fold higher than conventional ion exchangers
- Easy scale-up to production
- Belong to BioProcess™ Media family for perfomance and support to match their use in industrial downstream processing
- Q Sepharose XL virus licensed is identical to Q Sepharose XL in all aspects except for the addition of a license it provides for virus purification as described in the section "Important Information"
- All properties, applications work, etc described in this data file for Q Sepharose XL are also valid for Q Sepharose XL virus licensed

Amersham Biosciences

Characteristics

General

Sepharose XL ion exchangers result from a continuous program to develop chromatography media that fulfil the stringent demands of industrial applications.

With their extremely high loading capacities and excellent physical and chemical stability, they are ideal to use in the Capture stage of a downstream process which is characterised 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.

In addition, Q and SP Sepharose XL belong to the BioProcess Media family, which assures the process operator of a wide range of services tailor-made to support their use in commercial manufacture including regulatory support file, recommended columns and packing procedures.

Properties

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 this dextran through chemically stable ether bonds.

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

Table 1. Characteristics of Q Sepharose XL.

Type of ion exchanger	Strong anion
Ionic capacity	0.18–0.26 mmol Cl^-/ml adsorbent
Matrix structure	Cross-linked 6% agarose with bound dextran
Particle form	Spherical, 45–165 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 week, 20 °C) to pH <4 – Oxidizing agents
Physical stability	Negligible volume variation due to changes in pH or ionic strength
Recommended pH	
working range	2–12
cleaning-in-place	2–12
Recommended working flow rate	300–500 cm/h
Binding capacity[2]	>130 mg bovine serum albumin/ml adsorbent
Temperature stability	4 to 40 °C
Storage	20% ethanol

[1] No significant change in ionic binding capacity and carbon content after 1 week 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 flow rate 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	0.18–0.25 mmol H^+/ml adsorbent
Matrix structure	Cross-linked 6% agarose with bound dextran
Particle form	Spherical, 45–165 µm
Mean particle size	90 µm
Chemical stability[1] aqueous buffers	– Stable in all commonly used – 1 M NaOH – 20% ethanol – 6 M guanidine HCl Avoid: – Long exposure (1 week, 20 °C) to pH <4 – Oxidizing agents
Physical stability	Negligible volume variation due to changes in pH or ionic strength
Recommended pH	
working range	4–13
cleaning-in-place	4–13
Recommended working flow rate	300–500 cm/h
Binding capacity[2]	>160 mg lysozyme/ml adsorbent
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 week storage at 40 °C.
[2] Breakthrough capacity at 10% of 2 mg lysozyme/ml in a 4.4 ml packed bed at a linear flow rate of 300 cm/h in 50 mM Glycine HCl, pH 9.0, bed height 10 cm.

Very high loading capacity

The XL Extreme Load ion exchangers expose charged groups in an optimal fashion to bind target molecules. The dynamic binding capacity of Q and SP Sepharose XL is thus extremely high. Comparative studies have given breakthrough capacities that are up to 10 fold higher than other commercially available adsorbents.

Figures 2 and 3 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.

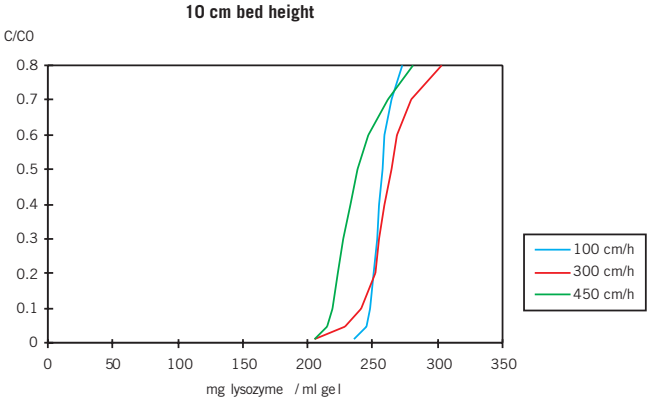


Fig 2. Dynamic binding capacity for lysozyme at different flow velocities on SP Sepharose XL packed in an XK 16/20 column at 10 cm bed height.

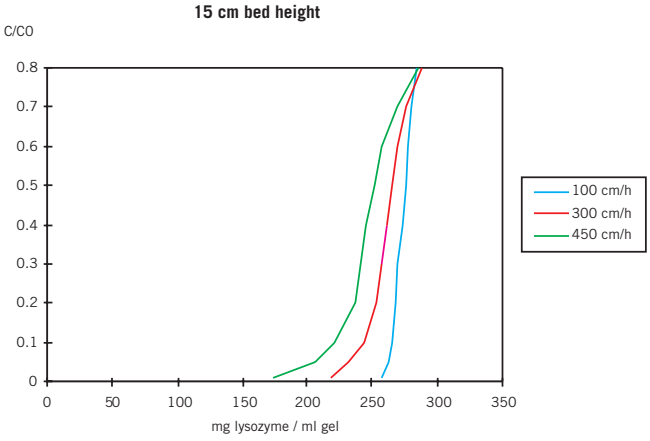


Fig 3. Dynamic binding capacity for lysozyme at different flow velocities on SP Sepharose XL packed in an XK 16/20 column at 15 cm bed height.

High resolution at high loading

The high binding capacity of Q 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 corresponding Sepharose Big Beads, Sepharose Fast Flow and Sepharose High Performance media. This similarity can speed up method development on Sepharose XL ion exchangers.

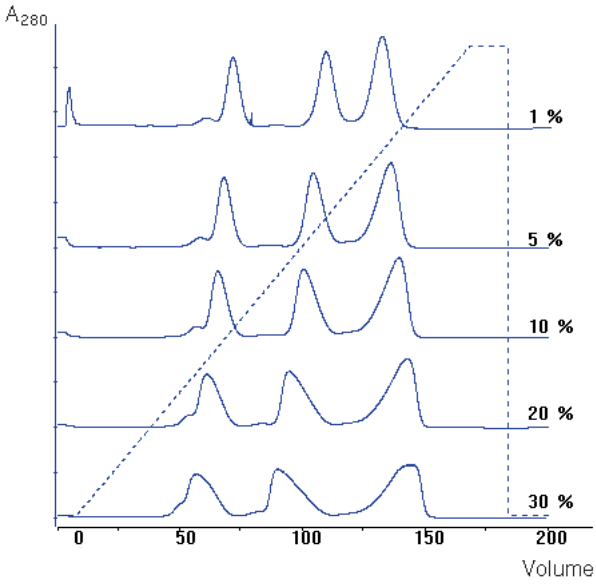


Fig 4. Separation of ribonuclease, cytochrome C and lysozyme on SP Sepharose XL. Increasing sample loading up to 10% has little effect on resolution. At 30% the peaks are broader, but resolution is still acceptable.

BioProcess Media – media made for bioprocessing

BioProcess Media are a specially selected range of separation media developed and supported for process scale chromatography. All media are produced following validated methods and are tested to ensure they meet the performance requirements of 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.

High chemical and mechanical stabilities give flexibility and long life

Q and SP Sepharose XL have high chemical stability and can be used over a wide pH range. This flexibility gives process operators considerable freedom when choosing adsorption and elution conditions, which helps them optimize the separation. In addition, effective cleaning and sanitization procedures can be applied, which extends the working life of the media and increases the overall economy of a process.

Operation

Scale up

Start by optimizing the separation at laboratory scale, 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 5–15 cm.

Equipment

Q 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 Amersham Biosciences.

Table 3. Recommended columns for Q and SP Sepharose XL.

Column	Inner diameter (mm)	Bed volume	Bed height (cm)
Lab-scale:			
XK 16/20	16	up to 30 mL	max. 15
XK 26/20	26	up to 80 mL	max. 15
Production scale:			
BPG™ 100/500	100	up to 2.4 litres	max. 30
BPG 200/500	200	up to 9.4 litres	max. 30
BPG 300/500	300	up to 21 litres	max. 30
BPG 450/500	450	up to 43 litres	max. 27
INdEX™ 70/500	70	up to 1.2 litres	max. 30
INdEX 100/500	100	up to 2.4 litres	max. 30
INdEX 200/500	200	up to 9.4 litres	max. 30
Chromaflow™ V400/100–300	400	^{2}37	30
Chromaflow V600/100–300	600	^{2}84	30
Chromaflow V800/100–300	800	^{2}150	30
Chromaflow V1000/100–300	1 000	^{2}236	30

Chromaflow columns are available even in larger diameters, please contact your Amersham Biosciences representative for information.

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

Application

Purification of recombinant α-amylase from E. coli. A scale up study

Recombinant α-amylase (MW 50 kDa) 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 done by method scouting in an XK 16/20 column run on ÄKTAexplorer™ 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 then 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 run with increasing amounts of starting material until a-amylase was detected in the flow through.

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 a-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 feed-stocks 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 is initial concentration and purification of the molecule of interest from crude or clarified feed-stock. Ion exchange is a technique well suited for Capture.

As the feed-stock may contain high levels of impurities including proteases, high loading and throughput are needed to ensure rapid processing and product stabilization prior to Intermediate Purification.

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 the a-amylase and that relatively little is found in the a-amylase peak. This 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 the α-amylase Captured at pilot scale.

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 CaCl2, centrifuged
Buffer A:	20 mM Tris-HCl, pH 8, 10 mM CaCl2
Buffer B:	20 mM Tris-HCl, pH 8, 1 M NaCl, 10 mM CaCl2
Flow rate:	300 cm/h, 12 L/h
Gradient:	20 bed volumes 0-1 M NaCl
Eluate:	1.48 L, 3.8 bed volumes
Spec. act. α-amylase	6420 U/L

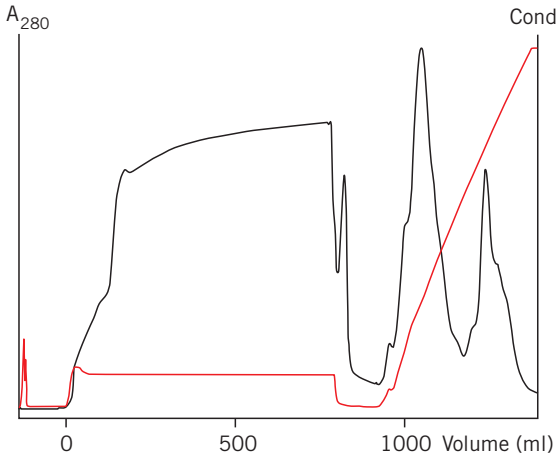
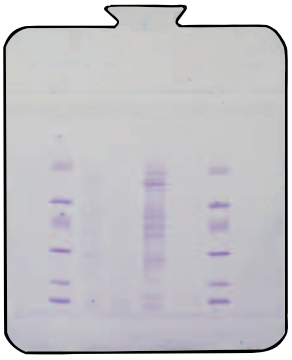


Fig 5. Pilot scale Capture of recombinant α-amylase from *E. coli* on Q Sepharose XL.



Lane 1: –
Lane 2: LMW markers.
Lane 3: Starting material.
Lane 4: Flow through.
Lane 5: 1st peak (containing α-amylase).
Lane 6: 2nd peak.
Lane 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.

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
Flow through	21.5
1st peak (containing α-amylase)	10
2nd peak	117

Ordering information

Product	Pack size	Code No.
Q Sepharose XL	300 mL	17-5072-01
	5 L	17-5072-04
	60 L	17-5072-60
Q Sepharose XL virus licensed	25 ml	17-5437-10
	300 ml	17-5437-01
	1L	17-5437-03
	5 L	17-5437-04
SP Sepharose XL	300 mL	17-5073-01
	5 L	17-5073-04
	60 L	17-5073-60

Q Sepharose XL and Q Sepharose XL virus licensed are supplied as a suspension in 20% ethanol. SP Sepharose XL is supplied as a suspension in 20% ethanol and 20 mM sodium acetate.

to order:

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