# Q and SP Sepharose big beads

ION EXCHANGE RESINS

Q and SP Sepharose<sup>™</sup> Big Beads are strong ion exchangers designed for industrial applications. The large particle size  $(d_{50v} \sim 200 \ \mu\text{m})$  and excellent physical stability of the base matrix ensure maintained speed even with viscous samples. Q and SP Sepharose Big Beads are therefore the ultimate ion exchange resins for initial purifications when high viscosity precludes the use of ion exchangers with smaller particle size, such as Sepharose Fast Flow ion exchangers. The unique flow characteristics are also invaluable when adsorption needs to be done quickly, e.g. in order to minimize proteolytic breakdown.

- Easy to scale-up
- High flow rates
- High chemical resistance for effective cleaning-in-place (CIP)
- Easy maintenance

## **Resin characteristics**

The ion exchange groups are coupled to the crosslinked agarose matrix through chemically stable ether bonds. The strong ion exchange groups maintain full protein binding capacity over the whole operating pH range. Q Sepharose Big Beads and SP Sepharose Big Beads have the same selectivities as the corresponding Sepharose Fast Flow and Sepharose High Performance ion exchangers.

#### **General maintenance**

Sepharose Big Beads ion exchange resins are easy to pack and handle. The very high flow rates that can be used save valuable time in equilibration and during regeneration. Even with viscosities as high as 2.5 times water a high flow rate (500 cm/h) is maintained in industrial column operation. Packed columns can be cleaned-and sanitized-in-place to minimize production losses.



Fig 1. Q and SP Sepharose Big Beads ion exchange resins.

#### Table 1. Characteristics of Q and SP Sepharose Big Beads

	Q Sepahrose Big Beads	SP Sepharose Big Beads	
Matrix	Cross-linked agarose, 6%, spherical		
Particle size, d <sub>500</sub> <sup>1</sup>	~ 200 µm	~ 200 µm	
lon exchange type	Strong anion	Strong cation	
lonic capacity	0.18–0.25 mmol Cl <sup>-</sup> /mL resin	0.18–0.25 mmol H*/mL resin	
Exclusion limit [M,] Globular proteins	~ 4 × 10 <sup>6</sup>	~ 4 × 10 <sup>6</sup>	
Pressure/Flow Specification <sup>2</sup>	1200 to 1800 cm/h at 0.1 MPa in a XK 50/60 column with 5 cm diameter and 25 cm bed height (at 20°C using buffers with the same viscosity as water)		
pH stability, operational <sup>3</sup>	2 to 12	4 to 13	
pH stability, CIP <sup>4</sup>	2 to 14	3 to 14	
pH ligand fully charged⁵	Entire pH range	Entire pH range	
Chemical stability	Stable to commonly used aqueous buffers, 1.0 M NaOH <sup>6</sup> , 70% ethanol, organic solvents		
Avoid	Oxidizing agents	Oxidizing agents	
Working temperature	4 to 30°C	4 to 30°C	
Storage	20% ethanol, 4 to 30°C	20% ethanol with 0.2 M sodium acetate, 4 to 30°C	

<sup>1</sup> Median particle size of the cumulative volume distribution.
<sup>2</sup> The pressure/flow characteristics describes the relationship between pressure and flow under the

<sup>2</sup> The pressure/flow characteristics describes the relationship between pressure and flow under the set circumstances. The pressure given shall not be taken as the maximum pressure of the resin.

<sup>3</sup> pH range where resin can be operated without significant change in function.

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

<sup>5</sup> 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.

<sup>6</sup> 1.0 M NaOH should only be used for cleaning purposes.









Fig. 3. Typical binding capacities of Sepharose Big Beads resins. The above shows the binding capacity of SP Sepharose Big Beads measured with frontal analysis in acetate pH 5 Bovine serum albumin (BSA) and formate pH 4.1 ( $\beta$ -lactoglobulin) at linear flow rates of 12 (corresponding to total available capacity) and 300 cm/h.

#### **Column packing**

Q and SP Sepharose Big Beads are easy to pack in small and large scale columns. Narrow peaks with high symmetry are reproducible whether you pack the ion exchanger bed with a constant pressure of between 1 to 3 bar, or let the slurry sediment and then compress it with the adaptor. Suction packing can easily be performed as well.

## Cleaning, sanitization, regeneration and storage

Due to the high chemical resistance of these resins, severe conditions can be used to clean and sanitize the column.

#### Cleaning-in-place

#### **Ionically bound proteins**

Wash with filtered 2 M NaCl at approximately 100 cm/h. Contact time: 10 to 15 min.

#### Hydrophobically bound proteins or lipoproteins

Wash with 1.0 M NaOH at 40 cm/h. Contact time: 1-2 h.

#### Lipids and very hydrophobic proteins

Wash with 70% ethanol at 40 cm/h, reversed flow, or with saw-tooth gradient 0-30-0% isopropanol. Contact time: 1-2 h.

#### Sanitization

A reduction of microbial contamination in the ion exchanger bed is obtained by washing the column with 0.5–1.0 M NaOH, allowing a contact time of 30-60 min.

#### Regeneration

Regeneration is performed by passing one bed volume of 1 M NaCl through the column. After regeneration, equilibrate the column with five column volumes of buffer.

#### Storage

Q and SP Sepharose Big Beads can be stored at neutral pH in 20% ethanol (Q Sepharose Big Beads) or in 20% ethanol with 0.2 M sodium acetate (SP Sepharose Big Beads). Alternative storage solution is 0.01 M NaOH\*.

\* In most cases, no long term stability data has been generated by Cytiva in 0.01 M NaOH. In some cases, accelerated studies at elevated temperature indicate that storage in 0.01 M NaOH can be a viable option but no guarantees can be made regarding retained function of the product.

### Operation

#### Equipment

Any standard chromatographic system from Cytiva can be used. Make sure the capacity of the pump is high enough to handle the very high flow rates used during column packing.

#### **Process optimization**

Normal optimization procedures for choosing buffer, ionic strength, pH, gradient shape and elution volume should be followed. The use of a higher bed height can give a better result due to the increased residence time.

## Ordering information

Product	Pack size	Code number
SP Sepharose Big Beads	1 L	17065703
	10 L	17065705
	60 L	17065760
Q Sepharose Big Beads	1 L	17098903
	10 L	17098905
	60 L	17065760

Pack sizes available upon request.

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