Q and SP Sepharose™ Big Beads

Q and SP Sepharose Big Beads are strong ion exchangers designed for industrial applications. The large particle size (100-300 µm) 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 media for initial purifications when high viscosity precludes the use of ion exchangers with smaller bead 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

Media characteristics

The ion exchange groups are coupled to the highly cross-linked 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 media 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. The media can also be autoclaved.

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

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.

Table 2. Characteristics of Q and SP Sepharose Big Beads

Property	Q	SP
Ion exchange type	Strong anion	Strong cation
Ionic capacity	180–250 µmole/ml gel	
Exclusion limit	4×10^6 daltons (globular proteins)	
Matrix	Macroporous, cross-linked agarose, 6%	
Bead form	Spherical, 100–300 µm	
Flow rate	1200–1800 cm/h*	
Working temp.	4–40°C	
pH stability	2-12 (working range)	4–12 (working range)
	2–14 (cleaning-in-place)	3–14 (cleaning-in-place)
Chemical stability	All commonly used aqueous buffers 1 M NaOH 70% ethanol Organic solvents	
The following should be avoided	Oxidizing agents Long exposure (1 week, 20°C) to pH <4	

* *25 cm bed height, 1 bar, 20°C distilled water in GE Healthcare XK 50 column.*

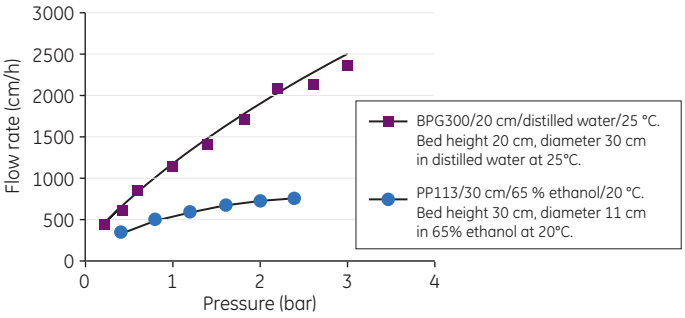

Fig. 2. The excellent flow characteristics of Sepharose Big Beads allow high flow rates even with high viscosity feed.

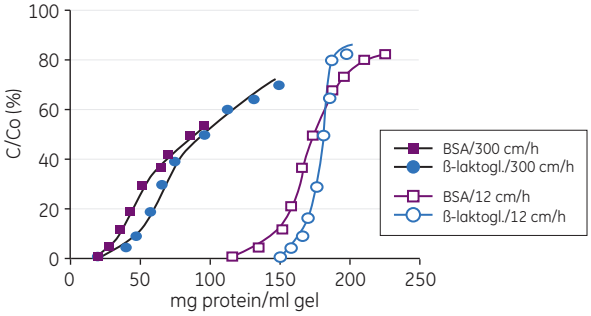

Fig. 3. Typical binding capacities of Sepharose Big Beads media. The above shows the binding capacity of SP Sepharose Big Beads measured with frontal analysis in acetate pH 5 Bovine serum albumin (BSA) and formate pH 4.1 (β-lactoglobulin) at linear flow rates of 12 (correspondmg to total available capacity)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

Cleaning, sanitization, regeneration and storage

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

Cleaning-in-place

Ionically bound proteins

Wash with filtered 2M NaCl at approximately 100 cm/h. Contact time: l0–15 min.

Hydrophobically bound proteins or lipoproteins

Wash with 1 M NaOH at 40 cm/h. Contact time: l–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: l–2 h.

Sanitization

A reduction of microbial contamination in the ion exchanger bed is obtained by washing the column with 0.5–l 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 either at neutral pH in buffer containing 20% ethanol or in 0.01 M NaOH.

Operation

Equipment

Any standard chromatographic system from GE Healthcare 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	17-0657-03
	10 L	17-0657-05
Q Sepharose Big Beads	1 L	17-0989-03
	10 L	17-0989-05

For local office contact information, visit
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