Sepharose 4 Fast Flow
Sepharose 6 Fast Flow

Sepharose™ 4 Fast Flow and Sepharose 6 Fast Flow gel filtration media are part of the Amersham Biosciences BioProcess™ Media range. BioProcess media are easy to scale up to routine commercial production since they perform well at all levels of operation and show high batch-to-batch reproducibility. All media in the BioProcess family are fully compatible with recommended procedures for process hygiene and maintenance, and meet the need for reliable, economic and safe production of biological material in large-scale operation.

- High chemical and physical stability
- Fast Flow characteristics
- Easy maintenance
- Scalable

Sepharose 4 Fast Flow and Sepharose 6 Fast Flow gel filtration media are based on highly cross-linked 4% and 6% agarose matrices, respectively, which give excellent physical stability and chromatographic qualities.

The matrices were developed specifically to meet the high throughput demands of industrial process separations. Their rigidity permits high flow rates, which in turn give good resolution in a minimum of time. Sepharose Fast Flow matrices enable the rapid processing of large volumes (1,2), making these media ideal for use at process-scale.

All Sepharose Fast Flow gel filtration media are easy to work with, and tolerate working conditions of temperature, pH and chemical agents typically used in biopharmaceutical production processes. Packed columns can be sanitized and cleaned-in-place (CIP) to minimize production losses through column maintenance. The media can be autoclaved, if required, for complete sterilization.

Fig 1. Sepharose 4 Fast Flow and Sepharose 6 Fast Flow for rapid process-scale gel filtration.

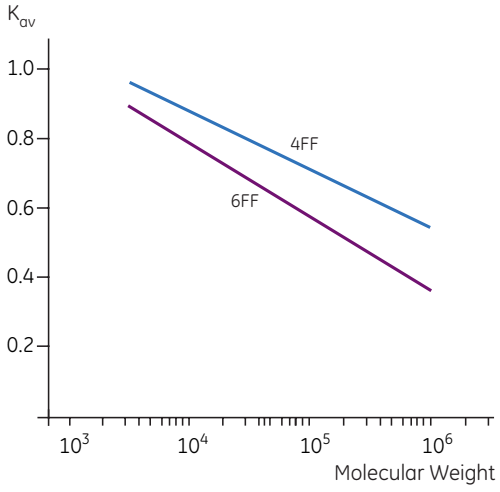

Fig 2. Selectivity curves for Sepharose 4 Fast Flow and Sepharose 6 Fast Flow (globular proteins).

Sepharose™

Table 1. Media characteristics.

	Sepharose 4 Fast Flow	Sepharose 6 Fast Flow
Exclusion limit		
for globular proteins	≈ 3×10^7	≈ 4×10^6
for dextrans	≈ 6×10^6	≈ 2×10^6
Mean particle size	90 µm	
Bead size range	45–165 µm	
Bead structure	highly cross-linked agarose, 4 or 6%, respectively, spherical	
Flow rate at 100 kPa, 25°C;	≥ 250 cm/h	≥ 300 cm/h
(XK 50/30 Column, 15 cm bed height)		
Recommended pH working range	2–12	
pH stability, CIP	2–14	
Chemical stability (40°C for 7 days in)	2 M NaOH	
	70% ethanol	
	30% isopropanol	
	30% acetonitrile	
	1% SDS	
	2% Asepto	
	6 M guanidine-hydrochloride	
	8 M urea	
Temperature stability (under storage conditions)	3–40 °C	
Autoclavable	121 °C for 20 minutes in H_2O	

Media characteristics

Operation and regeneration

Sepharose 4 Fast Flow and Sepharose 6 Fast Flow are supplied pre-swollen in 20% ethanol. Decant the excess ethanol and replace with working buffer before packing into columns. After packing, equilibrate the column with working buffer by washing with at least 5 bed volumes.

Stability

Sepharose 4 Fast Flow and Sepharose 6 Fast Flow have high chemical and mechanical stability. Table 1 summarizes their characteristics.

Process-scale use

Columns

A list of columns recommended for Sepharose 4 Fast Flow and Sepharose 6 Fast Flow can be found in Table 2. The operational flow rate should not exceed 80% of the packing flow rate.

Figure 3 shows pressure/flow curves for the media packed in a BPG™ 200 column. Details for packing the different columns can be found in each respective User Manual.

Application

Figure 4 describes how Sepharose Fast Flow media were used in the final polishing step in the purification of a recombinant hepatitis B surface antigen (r-HBsAg) from the cell culture supernatant of a transformed Chinese hamster ovary cell line (3).

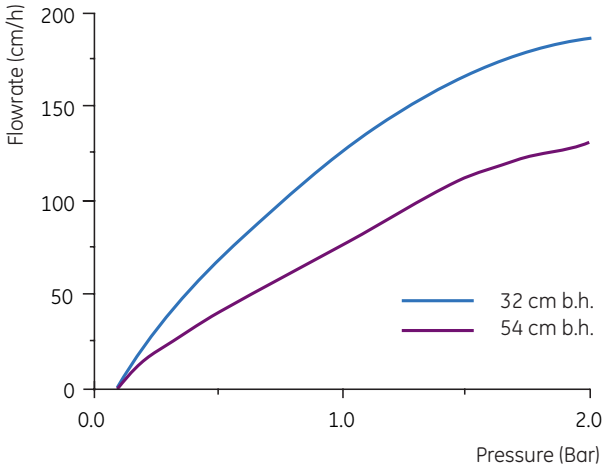

Fig 3. Pressure/flow rate curves for Sepharose 6 Fast Flow in a BPG 200 column, bed heights 32 cm and 54 cm, mobile phase H_2O.

After gradient PAGE analysis, the active fraction from the previous HIC and ion exchange steps showed the presence of significant amounts of relatively low molecular weight impurities which co-eluted with the r-HBsAg. By using gel filtration with Sepharose 4 Fast Flow, these impurities were efficienctly separated, especially with regard to the high molecular weight fraction A, which might contain residual plasmid or cellular DNA. Sepharose 6 Fast Flow is a more rigid medium, whereas the larger pore sizes of Sepharose 4 Fast Flow has advantages when purifying large proteins.

Process hygiene

Good process hygiene ensures the safety and integrity of the final product by removing or controlling any unwanted substances which might be present or generated in the raw material, or derived from the purification system itself. Good process hygiene also has a positive effect on process economy by preventing successive build-up of contaminating material on the separation media, thus increasing the life of the packed column.

Regeneration

Wash with 2 bed volumes of water, followed by 2–3 bed volumes of starting buffer. A complete cleaning-in-place (CIP) procedure is recommended after approximately 5 cycles, depending on the starting material.

Cleaning-in-place

Cleaning-in-place is the removal from the purification system of very tightly bound, precipitated or denatured substances generated in previous production cycles. In some applications, substances such as lipids or denatured proteins may remain in the column bed and not be eluted by the regeneration procedure. A specific CIP protocol has to be designed according to the type of contaminants known to be present in the feedstream. Recommended procedures for the removal of these contaminants without dismantling the column are described below. Column performance is not significantly changed by CIP procedures for at least 100 cycles.

1. Protocol to remove precipitated proteins:
   Wash the column with 4 bed volumes of 1.0–2.0 M NaOH solution at 40 cm/h, followed by 2–3 bed volumes of water.
2. Protocol to remove strongly bound hydrophobic proteins, lipoproteins and lipids:

- Wash the column with 4–10 bed volumes of up to 70% ethanol or 30% isopropanol. (Apply gradients to avoid air bubble formation when using high concentrations of organic solvents.)
- Alternatively, wash the column with detergent in a basic or acidic solution. For example, use 0.5% non-ionic detergent in 1.0 M acetic acid. Wash at a flow rate of 40 cm/h. Residual detergent can be removed by washing with 5 bed volumes of 70% ethanol.

For a further discussion of process hygiene, see reference 4

Sanitization and sterilization

Sanitization using NaOH reduces microbial contamination of the media bed to a minimum without dismantling the column. The CIP procedures recommended above also sanitize Sepharose Fast Flow media effectively: A concentration of 1.0–2.0 M NaOH with a contact time of 30–60 minutes has proved effective for most microbial contamination.

For sterilization of media, dismantle the column and autoclave at 121°C for 20 min. : Remember to sterilize the column parts before reassembling and packing the column.

Bed volume: 473 ml (XK 26/90 column)
Sample volume: 9.5 ml
Flow rate: 9 cm/h
Buffer: 20 mM Na_2HPO_4, 0.15 M NaCl, pH 7.0

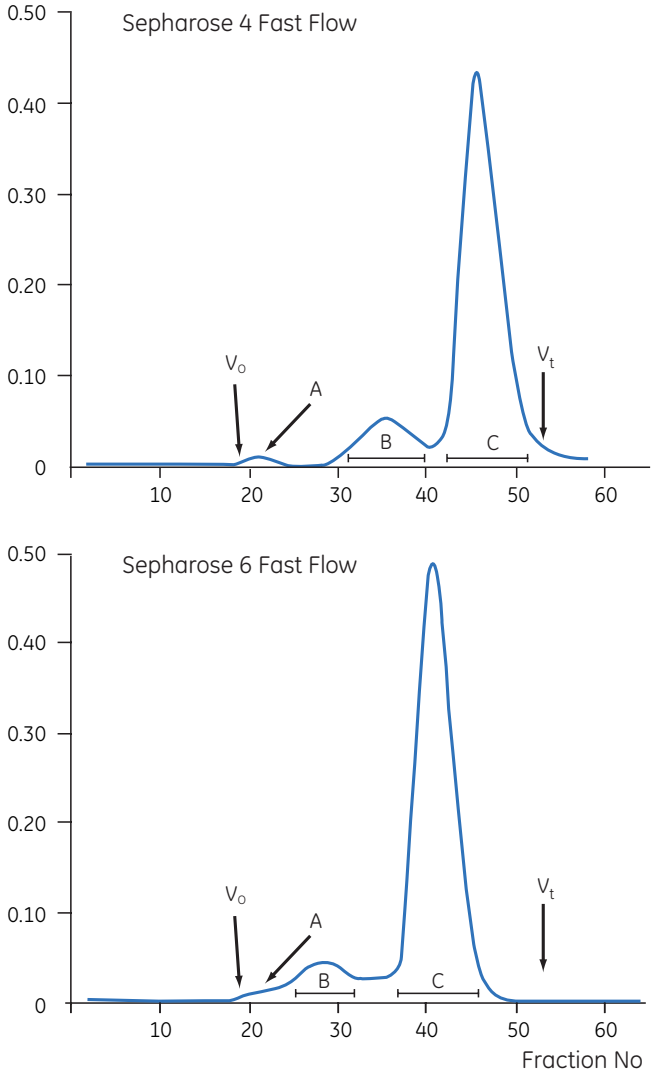

Fig 4. Polishing step in the purification of recombinant hepatitis B surface antigen.

Operation

After packing in the column, the bed should be washed with at least 3 column volumes of starting buffer to remove the preservative.

Storage

For longer periods of storage, e.g. weeks, we recommend that the media be stored at 3-8°C in 20% ethanol.

Use of Sepharose Fast Flow media as support for affinity ligands

Sepharose 4 Fast Flow and Sepharose 6 Fast Flow media can be used for the immobilization of ligands for affinity chromatography. The technique of immobilizing a ligand to a support matrix consists of three steps: First, activation of the matrix to make it reactive towards the functional group of the ligand. Next, coupling of the ligand.

Finally, deactivation or blocking of the residual active groups on the matrix. For detailed information on different experimental procedures, see reference 5. In general, the highly cross-linked Sepharose Fast Flow matrices need slightly stronger activation procedures than less cross-linked Sepharose media.

References

1. Hochuli, E. Large-scale recovery of Interferon - 2a synthesized in bacteria. *Chimia* **40**, 408-412 (1986).
2. Hochuli, E. Large-scale chromatography of recombinant proteins. *J. Chromatogr.* **444**, 293-302 (1988).
3. Belew, M. *et al.* Purification of recombinant hepatitis B surface antigen produced by transformed Chinese hamster ovary (CHO) cell line grown in culture. *Bioseparation* **1,** 397-408, (1991*)*.
4. Handbook of Process Chromatography. (1990) Academic Press Ltd., Sofer, G., Hagel, L.
5. Protein purification - principles, high resolution methods, and applications. Janson, J-C; Ryden, L., eds. 275-329, VCH Publishers Inc., (1989)

Ordering Information

Sepharose 4 Fast Flow and Sepharose 6 Fast Flow gel filtration media are supplied pre-swollen in 20% ethanol.

Product	Pack size	Code No
Sepharose 4 Fast Flow	1 1iter	17-0149-01
Sepharose 4 Fast Flow	5 liters	17-0149-04
Sepharose 4 Fast Flow	10 liters	17-0149-05
Sepharose 6 Fast Flow	1 liter	17-0159-01
Sepharose 6 Fast Flow	5 liters	17-0159-04
Sepharose 6 Fast Flow	10 liters	17-0159-05

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