GE Healthcare
Life Sciences

Sepharose™ 4 Fast Flow
Sepharose 6 Fast Flow

Sepharose 4 Fast Flow and Sepharose 6 Fast Flow gel filtration media (resins) are part of the GE Healthcare Life Sciences BioProcess™ media range (Fig 1). 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 good physical stability and chromatographic qualities. Figure 2 shows selectivity curves for globular proteins for the respective medium.

Sepharose Fast Flow matrices enable processing of large volumes, making these media suitable 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 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 processscale gel filtration.

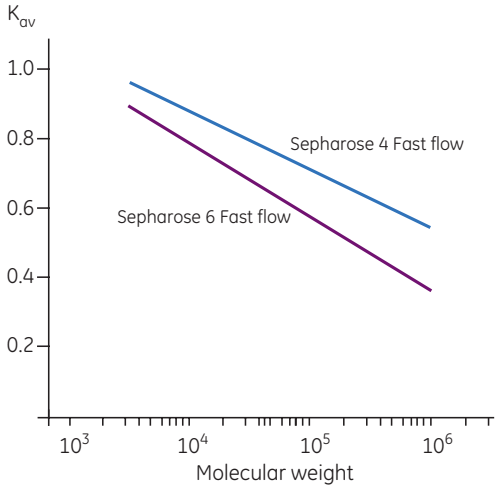

Fig 2. Selectivity curves for Sepharose 4 Fast Flow and Sepharose 6 Fast Flow (globular proteins).

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
Average particle size	90 µm	90 µm
Particale size range	45 to 165 µm	45 to 165 µm
Bead structure	highly cross-linked agarose, 4%	highly cross-linked agarose, 6%
Flow velocity at 100 kPa, 25°C; (XK 50/30 column, 15 cm bed height)	≥ 250 cm/h	≥ 300 cm/h
pH stability		
working range	2 to 12	2 to 12
cleaning-in-place	2 to 14	2 to 14
Chemical stability (40°C for 7 d)	2 M NaOH, 70% ethanol, 30% isopropanol, 30% acetonitrile, 1% SDS, 2% asepto, 6 M guanidine-hydrochloride, 8 M urea	
Autoclavable	121°C for 20 min in H_2O	121°C for 20 min in H_2O
Storage	20% ethanol at 4 to 30°C	20% ethanol at 4 to 30°C

Chromatography media characteristics

Operation and regeneration

Sepharose 4 Fast Flow and Sepharose 6 Fast Flow are supplied preswollen 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

The operational flow velocity should not exceed 80% of the packing flow velocity.

Figure 3 shows pressure/flow curves for Sepharose 6 Fast Flow packed in columns with bed heights 32 cm and 54 cm. 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.

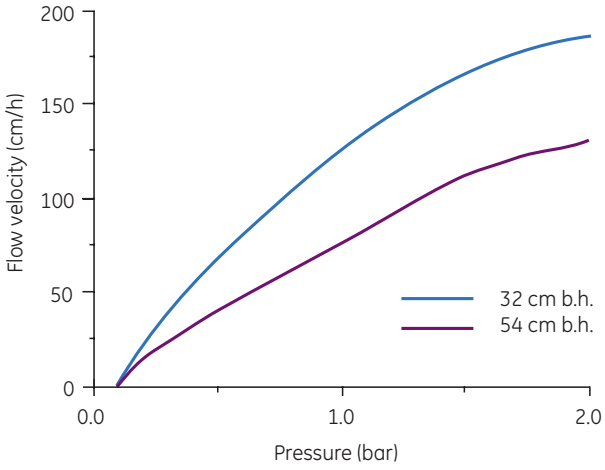

Fig 3. Pressure/flow velocity curves for Sepharose 6 Fast Flow in columns with bed heights 32 cm and 54 cm, mobile phase H_2O.

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 efficiently 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 to 3 bed volumes of buffer. A complete cleaning-in-place (CIP) procedure is recommended after approximately 5 cycles, depending on the starting material.

Cleaning-in-place

CIP is the removal 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 to 2.0 M NaOH solution at 40 cm/h, followed by 2 to 3 bed volumes of water.

2. Protocol to remove strongly bound hydrophobic proteins, lipoproteins, and lipids:

- Wash the column with 4 to 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% nonionic detergent in 1.0 M acetic acid. Wash at a flow velocity of 40 cm/h. Residual detergent can be removed by washing with 5 bed volumes of 70% ethanol.

Sanitization

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 to 2.0 M NaOH with a contact time of 30 to 60 min has proved effective for most microbial contamination.

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

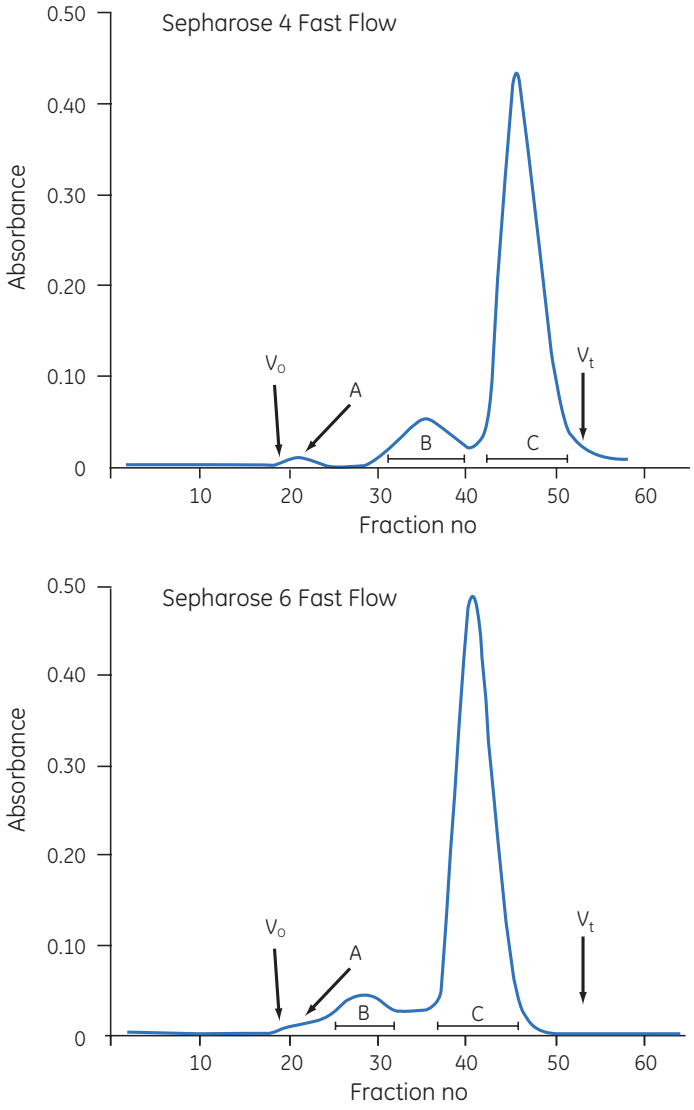

Fig 4. Polishing step in the purification of recombinant hepatitis B surface antigen.

Column efficiency testing

After packing the column, a test of the column efficiency to check the quality of packing should be performed. Testing should be performed immediately after packing and at regular intervals during the working life of the column and also when separation performance is seen to deteriorate.

Operation

The bed should be washed with at least 3 bed volumes of buffer to remove the preservative.

Storage

20% ethanol at 4 to 30°C.

Ordering information

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

Product	Pack size	Code number
Sepharose 4 Fast Flow	1 L	17-0149-01
Sepharose 4 Fast Flow	10 L	17-0149-05
Sepharose 6 Fast Flow	1 L	17-0159-01
Sepharose 6 Fast Flow	10 L	17-0159-05

For local office contact information, visit
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