Sepharose™ 4 Fast Flow
Sepharose 6 Fast Flow

SIZE EXCLUSION CHROMATOGRAPHY

Sepharose™ 4 Fast Flow and Sepharose 6 Fast Flow size exclusion chromatography resins are part of the Cytiva BioProcess™ resin range (Fig 1). BioProcess resins 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 resins 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 size exclusion chromatography resins are based on 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 resin.

Sepharose Fast Flow matrices enable processing of large volumes, making these resins suitable for use at process scale.

All Sepharose Fast Flow size exclusion chromatography resins 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 resins can be autoclaved, if required, for complete sterilization.

Fig 1. Sepharose 4 Fast Flow and Sepharose 6 Fast Flow for rapid process scale size exclusion chromatography.

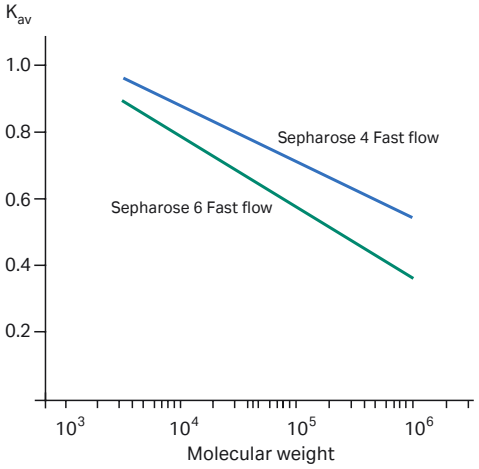

Fig 2. Selectivity curves for Sepharose 4 Fast Flow and Sepharose 6 Fast Flow (globular proteins).

Table 1. Characteristics of Sepharose 4 Fast Flow and Sepharose 6 Fast Flow

	Sepharose 4 Fast Flow	Sepharose 6 Fast Flow
Matrix	Cross-linked agarose, 4%, spherical	Cross-linked agarose, 6%, spherical
Particle size, d_{50v} [1]	~ 90 µm	~ 90 µm
Exclusion limit [M_r]		
Globular proteins	~ 3×10^7	~ 4×10^6
Dextrans	~ 6×10^6	~ 2×10^6
Pressure/flow characteristics[2]	150 to 250 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).	250 to 400 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[3]	3 to 13	3 to 13
pH stability, CIP[4]	2 to 14	2 to 14
Chemical stability	Stable to commonly used aqueous buffers, 2.0 M NaOH[5], 70% ethanol, 30% isopropanol, 30% acetonitrile, 1% SDS, 2% asepto, 6 M guanidine hydrochloride, 8 M urea	
Autoclavability	20 min at 121°C in H_2O	20 min at 121°C in H_2O
Storage	20% ethanol, 4°C to 30°C	20% ethanol, 4°C to 30°C

[1] Median particle size of the cumulative volume distribution.
[2] 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.
[3] pH range where resin can be operated without significant change in function.
[4] pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.
[5] 2.0 M NaOH should only be used for cleaning purposes.

Chromatography resin 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 resins 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.

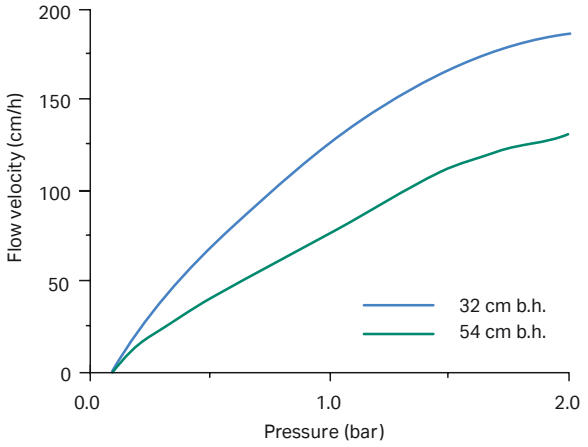

Fig. 3 Pressure/flow velocity curves for Sepharose 6 Fast Flow resin in a BPG 200 column with bed heights 32 cm and 54 cm, mobile phase water, and at room temperature.

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 size exclusion chromatography 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 resin, 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 resins, 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 resin bed to a minimum without dismantling the column. The CIP procedures recommended above also sanitize Sepharose Fast Flow resins 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

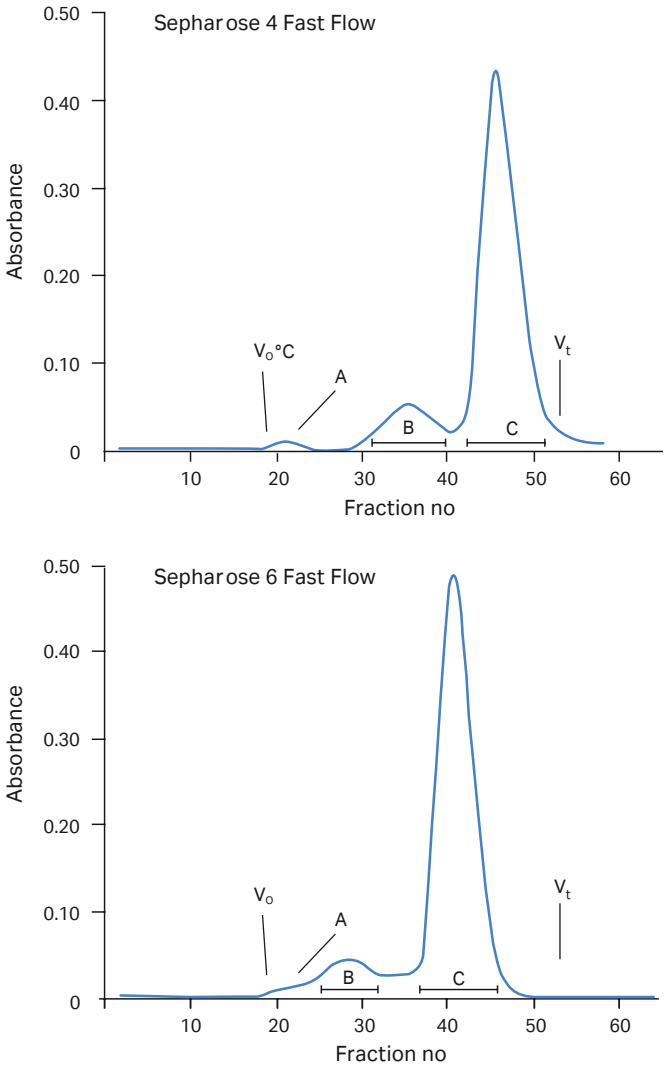

Fig 4. Polishing step in the purification of recombinant hepatitis B surface antigen.

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°C to 30°C.

Ordering information

Sepharose 4 Fast Flow and Sepharose 6 Fast Flow size exclusion chromatography resins are supplied preswollen in 20% ethanol.

Product	Pack size	Product code
Sepharose 4 Fast Flow	1 L	17014901
Sepharose 4 Fast Flow	10 L	17014905
Sepharose 6 Fast Flow	1 L	17015901
Sepharose 6 Fast Flow	10 L	17015905

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