Butyl **Sepharose** 4 Fast Flow

BIOPROCESS RESIN

Butyl Sepharose™ 4 Fast Flow is part of Cytiva's range of resins for hydrophobic interaction chromatography (HIC) and is designed for rapid processing of large volumes early in the downstream purification process. Butyl Sepharose 4 Fast Flow is a BioProcess™ resin and carries comprehensive technical and regulatory support for production scale applications.

- High dynamic capacity even at low salt concentrations
- Excellent flow characteristics
- No charged groups, making true hydrophobic interaction chromatography possible, without interfering ionic effects
- Specially developed in co-operation with commercial pharmaceutical manufacturers
- Easy scale-up

Fig 1. Butyl Sepharose 4 Fast Flow — for rapid purification.

Resin characteristics

Butyl Sepharose 4 Fast Flow is based on cross-linked 4% agarose. The aliphatic ligand is immobilized to the base matrix with an ether linkage. The ligand contains no charged groups, making true hydrophobic interaction chromatography possible, without interfering ionic effects. The Fast Flow matrix gives the adsorbent high chemical, physical and thermal stabilities, making this resin ideal for initial Capture and Intermediate Purification stages of downstream processing.

Stability

Butyl Sepharose 4 Fast Flow can be used with aqueous buffers commonly used in chromatography. The resin has high chemical and physical stabilities and withstands high concentrations of denaturing agents such as guanidine hydrochloride. It also has high thermal stability and can be autoclaved at 121°C for 20 min repeatedly. Table 1 summarizes its characteristics.

Table 1. Characteristics of Butyl Sepharose 4 Fast Flow

Matrix	Cross-linked agarose, 4%, spherical
Type of ligand	Butyl: R-O-CH_2-CH(OH)-CH_2-O-$(CH_2)_3$-CH_3
Particle size, d_{50v}[1]	~ 90 µm
Ligand concentration	~ 40 µmol Butyl/mL resin
pH stability, operational[2] pH stability, CIP[3]	3 to 13 2 to 14
Chemical stability	Stable to commonly used aqueous buffers, 1 mM HCl, 1.0 M NaOH[4], 30% isopropanol, 70% ethanol, 6 M guanidine hydrochloride
Autoclavability	20 min at 121°C in distilled water, 5 cycles
Pressure/flow characteristics	150–250 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)[5,6]
Operating temperature	4°C to 40°C
Delivery conditions	20% ethanol

[1] Median particle size of the cumulative volume distribution.
[2] pH range where resin can be operated without significant change in function.
[3] pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.
[4] 1.0 M NaOH should only be used for cleaning purposes
[5] 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.
[6] Pressure/flow test performed on the base matrix.

Operation

Optimization

HIC for proteins is usually performed in the presence of moderately high concentrations of anti-chaotropic salts. Substances are separated on the basis of their varying strength of hydrophobic interactions with hydrophobic groups immobilized to an uncharged resin matrix.

Several factors influence the chromatographic behavior of proteins and peptides in HIC. Some of the factors are crucial for developing a purification procedure. However, purification protocols for analytical separations differ from those for preparative separations and consequently the importance of the parameters varies with the type of purification. Scale of operation also influences the purification protocol. At small scale, the emphasis is usually on resolution whereas in large scale manufacturing, the emphasis is on throughput or productivity.

Parameters that impact binding, resolution, selectivity and recovery are:

- Sample characteristics
- Flow rate
- Salting-out effect
- Ionic strength
- Temperature
- pH

For Butyl Sepharose 4 Fast Flow, dynamic binding capacity decreases with increasing flow rate, a dependence that is also affected by salt concentration and the type of buffer ion used. To obtain the highest capacity and productivity, the contact time of the sample in the column should be optimized.

The advantage of using HIC is that it is compatible with other chromatographic techniques commonly used in purification processes. Ammonium sulphate precipitation, ion exchange and some affinity chromatography methods leave samples in a high salt concentration. Even after a step like this, the sample can often be directly transferred to a HIC column without the need for desalting with dialysis or size exclusion chromatography. HIC is also practical to use as the step before techniques like size exclusion chromatography, ion exchange, reversed phase, and affinity chromatography since elution of the protein of interest from a HIC column normally leaves the substance in a low ionic strength environment.

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 runs. Figure 3 shows the results of a clearance study performed at two different flow rates; this is a test which determines the amount of water required to remove the 20% ethanol.

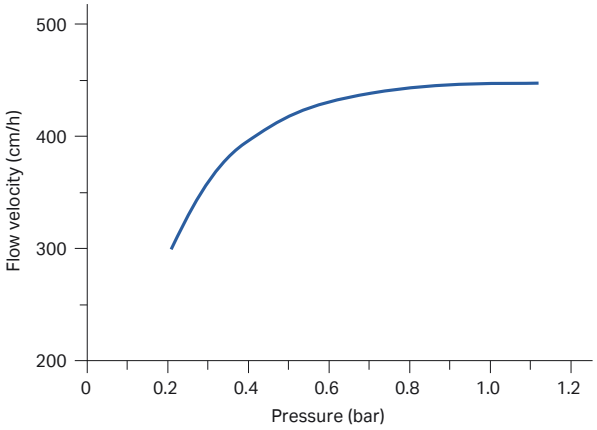


Fig 2. Pressure/flow curve for Butyl Sepharose 4 Fast Flow in an XK 50/30 column, bed height 150 mm, mobile phase 0.1 M NaCl (work from Cytiva).

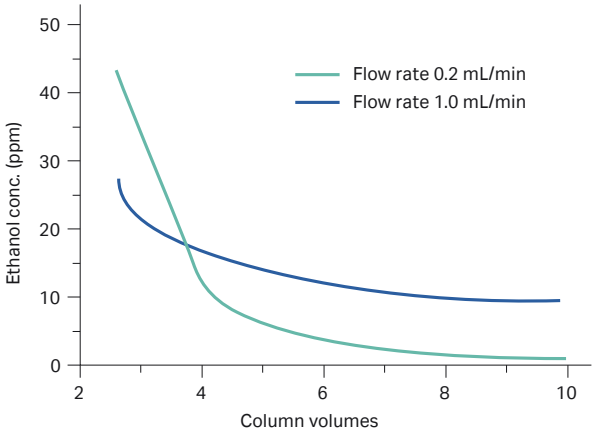


Fig 3. Removal of 20% ethanol from Butyl Sepharose 4 Fast Flow packed in an HR 10/10 column, bed volume 8 mL, mobile phase water.

Cleaning-in-place

Cleaning-in-place (CIP) removes very tightly bound, precipitated or denatured substances generated during previous production runs. In some applications, substances like lipids or denatured proteins may remain in the column bed and not be eluted by the regeneration procedure. CIP protocols should therefore be developed for the type of contaminants known to be present in the feedstream. Recommended procedures for removing specific contaminants are described below. CIP procedures can usually be carried out for hundreds of cycles without affecting column performance.

Suggested protocol to remove precipitated proteins:

- Wash the column with 4 bed volumes of 0.5–1.0 M NaOH solution at 40 cm/h, followed by 2–3 bed volumes of water

Suggested protocol to remove tightly 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. 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 is the use of chemical agents to inactivate microbial contaminants. Sodium hydroxide (NaOH) is a commonly used sanitizing agent. A concentration of 0.5–1.0 M NaOH with a contact time of 30–60 min is effective for most microbial contamination.

Sterilization

To sterilize Butyl Sepharose 4 Fast Flow, dismantle the column and autoclave the resin at 121°C for 20 min. Remember to sterilize the column parts before reassembling and re-packing.

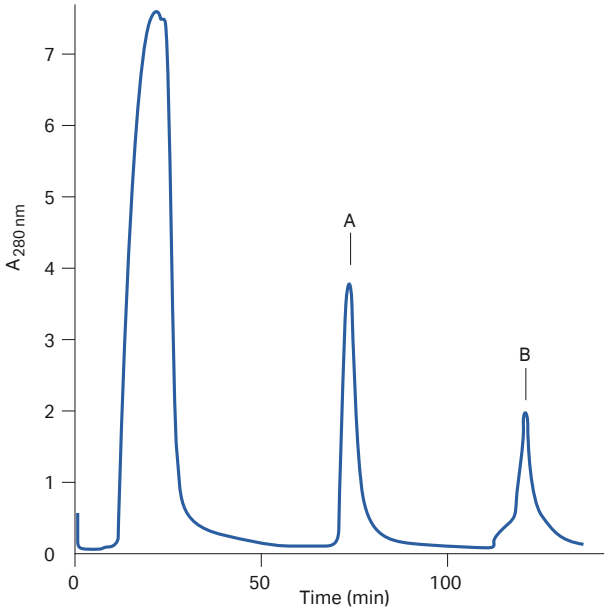
Storage

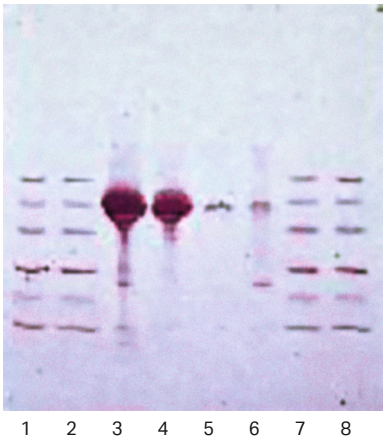
For long storage periods (weeks), the resin can be stored in 20% ethanol at a temperature of 4°C to 30°C . Butyl Sepharose 4 Fast Flow is supplied in 20% ethanol.

Application

A cell culture supernatant from transformed Chinese hamster ovary (CHO) cells was applied to Butyl Sepharose 4 Fast Flow. The chromatogram in Figure 4 shows the peaks obtained using two different elution buffers.

Resin:	Butyl Sepharose 4 Fast Flow
Sample:	Cell culture supernatant from transformed CHO cells
Column:	XK 16/20, 12 cm bed height
System:	BioPilot System
Loading:	40 mL supernatant
Starting buffer:	0.5 M $(NH_4)_2SO_4$ + 50 mM Na_2HPO_4, pH 7.0
Elution buffer A:	20 mM Na_2HPO_4, pH 7.0
Elution buffer B:	20 mM Na_2HPO_4, 30% isopropanol
Electrophoresis:	PhastGel™ 8–25% PAA, SDS, Coomassie™ staining





Lanes

1. Low molecular weight markers
2. Low molecular weight markers
3. Sample
4. Flow-through material
5. Buffer B eluate
6. Buffer C eluate
7. Low molecular weight markers
8. Low molecular weight markers

Fig 4. Example of the separation pattern of a cell culture supernatant after a one-step chromatographic separation on Butyl Sepharose 4 Fast Flow.

References

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Ordering information

Product	Pack size	Code number
Butyl Sepharose 4 Fast Flow	25 mL	17098010
Butyl Sepharose 4 Fast Flow	200 mL	17098001
Butyl Sepharose 4 Fast Flow	500 mL	17098002
Butyl Sepharose 4 Fast Flow	5 L	17098004
Butyl Sepharose 4 Fast Flow	10 L	17098005

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