

Butyl Sepharose® 4 Fast Flow BioProcess™ Media

Data File

Hydrophobic Interaction Chromatography

Butyl Sepharose® 4 Fast Flow is part of the Amersham Biosciences media range 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 medium 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

Media characteristics

Butyl Sepharose 4 Fast Flow is based on highly 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 medium ideal for initial Capture and Intermediate Purification stages of downstream processing.

Stability

Butyl Sepharose 4 Fast Flow can be used with all aqueous buffers commonly used in chromatography. The medium 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 minutes repeatedly. Table 1 summarizes its characteristics.



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

Table 1. Media Characteristics.

Degree of substitution	approx. 50 μmol butyl/mL gel
Mean particle size	90 μm
Bead size range	45–165 μm
Bead structure	highly cross-linked 4%, agarose, spherical
Linear flow rate at 25°C XK 50/60 Column, 25 cm bed height in water	≥ 150 cm/h at 100 kPa (1 bar, 14.5 psi)
pH stability working range at 25°C	3–13
cleaning (CIP)	2–14
Chemical stability 40°C for 7 days in:	1M NaOH 1 mM HCl 70% ethanol 30% isopropanol 6 M guanidine hydrochloride
Temperature stability under storage conditions	3–40°C
Autoclavable	121°C for 20 minutes in water

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 gel matrix.

Several factors influence the chromatographic behaviour 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 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 gel filtration, 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.

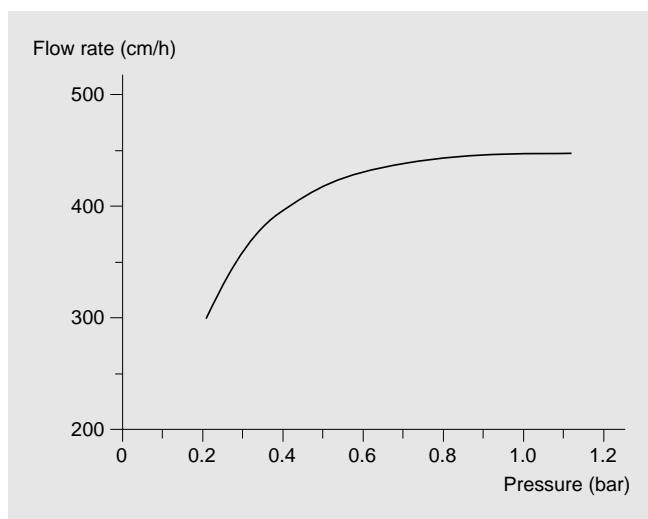


Fig. 2. Pressure/flow rate curve for Butyl Sepharose 4 Fast Flow in an XK 50/30 column, bed height 150 mm, mobile phase 0.1 M NaCl (work from Amersham Biosciences AB).

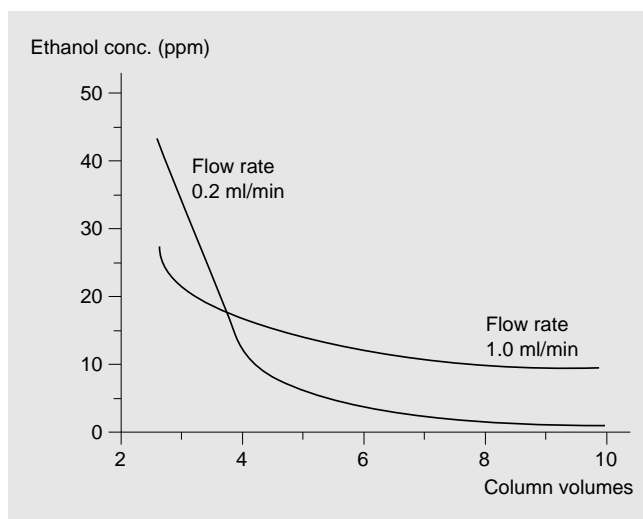


Fig. 3. Removal of 20% ethanol from Butyl Sepharose 4 Fast Flow packed in an HR 10/10 column, bed volume 8 mL, mobile phase water.

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, for example 0.5% non-ionic detergent in 1 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.

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 medium at 121°C for 20 min. Remember to sterilize the column parts before reassembling and re-packing.

Storage

For long storage periods (weeks), we recommend that the medium be stored in 20% ethanol at a temperature of +4–+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.

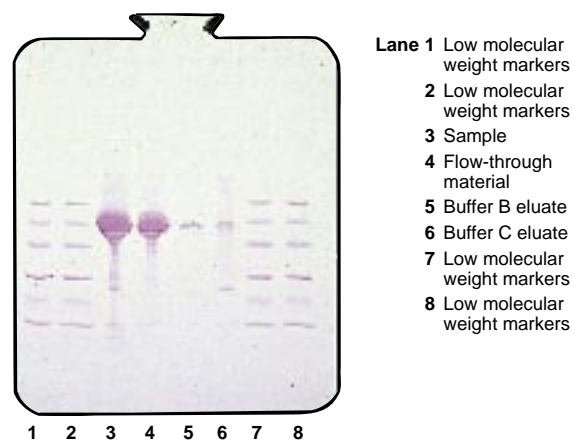
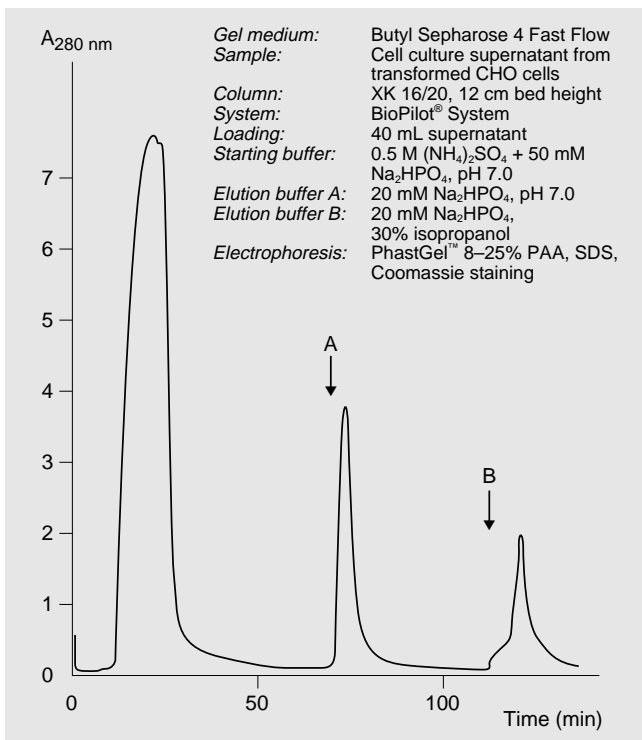


Fig. 4. Example of the separation pattern of a cell culture supernatant after a one-step chromatographic separation on Butyl Sepharose 4 Fast Flow.

Ordering Information

Product	Code No.	Pack size
Butyl Sepharose 4 Fast Flow	17-0980-01	200 mL
Butyl Sepharose 4 Fast Flow	17-0980-02	500 mL
Butyl Sepharose 4 Fast Flow	17-0980-04	5 L

References

- Specific salt effects in hydrophobic interaction chromatography of proteins. *Chromatographia* 28 (1988) 13–18, Szepesy, L., Horvath, C.
- Sofer G. K., Nyström L.-E., eds. Process Chromatography – a practical guide. London: Academic Press Ltd, 1989.
- Sofer G. K., Nyström L.-E., eds. Process Chromatography – a guide to validation. London: Academic Press Ltd, 1991.
- Functional, chemical and physical characterization of a new BioProcess medium for hydrophobic interaction chromatography (HIC) Butyl Sepharose 4 Fast Flow. Poster presented at: Recovery of Biological Products VI. An Engineering Foundation Conference, Interlaken, Switzerland, 20–25 Sept., 1992. Berggrund, A., *et al.*
- Gradient and isocratic high-performance hydrophobic interaction chromatography of proteins on agarose columns. *J. Chromatogr.* 359 (1986) 99–109, Hjertén, S., Yao, K., Eriksson, K.-O.; Johansson, B.
- Hydrophobic interaction high-performance liquid chromatography of proteins. *J. Chromatogr.* 317 (1984) 55–65, Goheen, S. C., Engelhorn, S. C.
- Interplay of hydrophobic and electrostatic interactions in biopolymer chromatography. Effect of salts on the retention of proteins. *J. Chromatogr.* 469 (1989) 3–27, Melander, W. R., El Rassi, Z., Horvath, C.
- Hydrophobic interaction chromatography of proteins on Separon HEMA. I. The effect of an initial salt concentration on the separation of proteins. *J. Chromatogr.* 479 (1989) 327–334, Kleinmann, I., Plicka, J., Smidl, P., Svoboda, V.
- von Hippel, P. H., Schleich, T. The effects of neutral salts on the structure and conformational stability of macromolecules in solution. In: eds., Timasheff S. N., Fasman, G. D. Structure and stability of biological macromolecules. New York: Marcel Dekker Inc., 1974, 417–574.
- Chromatography of lipophilic proteins on adsorbents containing mixed hydrophobic and ionic groups. *Biochem. J.* 126 (1972) 765–769, Yon, R. J.
- Salting-out in amphiphilic gels as a new approach to hydrophobic adsorption. *Nature* 245 (1973) 465–466, Porath, J.
- Some general aspects of hydrophobic interaction chromatography. *J. Chromatogr.* 87 (1973) 325–331, Hjertén, S.
- Hydrocarbon-coated Sepharoses: Use in the purification of glycogen phosphorylase. *Biochem. Biophys. Res. Commun.* 49 (1972) 383–390, Er-el, Z., Zaidenzaig, Y., Shaltiel, S.
- On the mode of adsorption of proteins to hydrophobic columns. *Biochem. Biophys. Res. Commun.* 72 (1976) 108–113, Wilchek, M., Miron, T.

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