

Butyl Sepharose 4 Fast Flow

Product Information

Cat#No# Bu-410C

Product Overview

Butyl Sepharose 4 Fast Flow is a well established, standard aliphatic hydrophobic interaction chromatography (HIC) resin for capture and intermediate purification of larger proteins.

Description

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.

Characteristic

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.

Maximum operating pressure

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)

Sample preparation

The sample must be dissolved in start buffer. Alternatively the sample can be transferred to start buffer by dialysis or by buffer exchange using a HiTrap Desalting or a PD-10 Desalting column. The viscosity of the sample must not exceed that of the buffer. For normal aqueous buffer systems, this corresponds to a protein concentration of approximately 50 mg/mL.

Before application the sample must be centrifuged or filtered through a 0.45 µm filter to remove any particulate matter.

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Packing Column

1. Pour the resin slurry into the column in one continuous motion. Pouring down a glass rod held against the wall of the column helps prevent the introduction of air bubbles. Fill the remainder of the column with buffer.
 2. Wet the column adapter by submerging the plunger end in buffer, and drawing buffer through with a syringe or pump. Make sure that all bubbles have been removed. Disconnect the pump or syringe.
 3. Insert the adapter into the top of the column at an angle, taking care not to trap air under the net. Tighten the adapter O-ring to give a sliding seal on the column wall.
 4. Fit a syringe barrel to the sample application valve and connect the valve between the adapter and the pump.
 5. Open the bottom outlet of the column and start the pump.
 6. Stop the pump, close the column outlet, loosen the adapter O-ring to give a sliding seal and reposition the adapter on the surface of the resin bed. Press the adapter into the surface of the resin an additional 1 to 2 mm.
 7. Lock the adapter in position, open the column outlet and start the pump at the column packing flow rate.
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Matrix

cross-linked agarose, 4%, spherical

Average particle size

~90 μm

Ligand

Butyl

Ligand density

~40 μmol Butyl/mL resin

Recommended column height

25 cm

Chemical stability

Stable in commonly used aqueous buffers - 1.0 M NaOH, 30% isopropanol, 70% ethanol, 6 M guanidine-hydrochloride, 30% Acetonitrile, 1mM HCl.

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pH working range

3–13

CIP stability

2–14

Temperature stability

4°C to 40°C

Autoclavable

20 min at 121 °C, 5 cycles in H₂O

Storage

4 to 30°C, 20% Ethanol

Shipping

20% ethanol

Evaluation of Packing

The best method of expressing the efficiency of a packed column is in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor (As). The values are easily determined by applying a test sample such as 1% acetone solution or sodium chloride to the column.

Binding

Salts that cause salting-out (for example ammonium sulphate) also promote binding to hydrophobic ligands. The column is equilibrated and the sample is applied in a solution of high ionic strength. A typical starting buffer is 1.7 M (NH₄)₂SO₄ , which is just below the concentration employed for salting out proteins. Hydrophobic interactions are weaker at lower temperatures. This must be taken into account if chromatography is performed in a cold room.

Equilibration

To equilibrate, pump approximately 100 mL of start buffer through the column at a flow rate of 2.5 mL/min. The column is fully equilibrated when the pH and/or conductivity of the effluent is the same as the start buffer.

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Elution

Bound proteins are eluted by reducing the strength of the hydrophobic interaction. This can be done by:
Reducing the concentration of salting-out ions in the buffer with a decreasing salt gradient (linear or step).
Increasing the concentration of chaotropic ions in the buffer with an increasing gradient (linear or step).
Eluting with a polarity-reducing organic solvent (for example ethylene glycol) added to the buffer.
Eluting with detergent added to the buffer.

Regeneration

For best performance from the resins, bound substances must be washed from the column after each chromatographic cycle. Wash with 2 bed volumes of water, followed by 2 to 3 bed volumes of starting buffer.

Cleaning-in-place

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.
Remove tightly bound hydrophobic proteins, lipoproteins and lipids: Wash the column with 4–10 bed volumes of up to 70% ethanol or 30% isopropanol. 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.

Pack size

200 mL

BioProcess resin

Yes

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Dimensions

5 cm
