Butyl Sepharose High Performance

Product Information

Cat#No#	Bu-413C

Product Overview

Butyl Sepharose High Performance is an aliphatic hydrophobic interaction chromatography (HIC) medium, designed for intermediate and polishing step purification steps when high resolution has priority.

Description

Butyl Sepharose High Performance is a member of the Cytiva range of HIC resins for intermediate purification and polishing of proteins. Butyl Sepharose High Performance is based on highly crosslinked, 34-µm agarose beads modified with aliphatic butyl groups via uncharged, chemically stable ether linkages. It is a truly hydrophobic resin displaying a minimum of ionic interactions. Butyl Sepharose High Performance resins is particularly well suited for intermediate purification and polishing steps providing high resolution due to the small particle size.

Characteristic

High-resolution, high-capacity separations with high recovery.
Reliable and reproducible.
High chemical stability for effective CIP and sanitization.
Available in laboratory and BioProcess scale quantities.
Easy to scale up.

Maximum operating pressure

Base matrix: 100-200 cm/h, 300 kPa, BioPilot 60/600 column, bed height 30 cm.

Matrix

cross-linked agarose

Particle Size

24 µm-44 µm

Average particle size

~34 µm

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CREATIVE BIOMART®
Chromatography

Butyl Sepharose High Performance

Ligand
Butyl
Ligand density
Approx. 50 µmol butyl/ml gel
Dynamic binding capacity
~ 38 mg β-lactoglobulin/mL resin
Recommended flow rate
≤ 100 cm/h
Recommended column height
30 cm
Chemical stability
Stable in commonly used aqueous buffers - 1 M sodium hydroxide, 1 M acetic acid, 8 M urea, 6 M guanidine hydrochloride, 30% acetonitrile, 70% ethanol, 3 M ammoniumsulfate, 1 mM HCl, 30% isopropanol, 2% SDS
pH working range
3–13
CIP stability
2–14
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). These values are easily determined by applying a sample such as 2.0 M NaCl in water with 0.5 M NaCl in water as eluent. A solution of acetone (1%) in water can also be used as a test substance, but can interact with the hydrophobic resin.
Regeneration
For best performance from the resin, wash bound substances from the column after each chromatographic cycle. Wash with 2 bed volumes of water, followed by 2 to 3 bed volumes of starting buffer.

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Cleaning-in-place

Removal of precipitated proteins: Wash the column with 4 bed volumes of 0.01 M NaOH at 40 cm/h, followed by 2 to 3 bed volumes of water.
Removal of tightly bound hydrophobic proteins, lipoproteins and lipids: Wash the column with 4 to 10 bed volumes of up to 70% ethanol or 30% isopropanol followed by 3 to 4 bed volumes of water.
Alternatively, wash the column with detergent in a basic or acidic solution, for example, 0.5% nonionic detergent in 1 M acetic acid. Wash at a flow velocity of 40 cm/h. Remove residual detergent with 5 bed volumes of 70% ethanol followed by 3 to 4 bed volumes of water.

Sanitization

Wash the column with 0.01 M NaOH at a flow velocity of approximately 40 cm/h, contact time 30 to 60 min.

Scaling up

1. Select the bed volume according to required binding capacity.
2. Select a column diameter to obtain a bed height of 10 to 25 cm.
3. While keeping bed height and flow velocity constant, increase bed diameter and volumetric flow rate.

Purification procedures

1. Add the salt dissolved in a neutral buffer to the feed stock until the predetermined concentration is reached. The exact salt concentration must determined for each target molecule.
2. Equilibrate the column with start buffer of the same salt concentration as in the feed.
3. Apply the sample to the column.
4. Wash out unbound sample using start buffer.
5. Elute the target protein by applying a gradient of descending concentration of salt. Typically, the gradient is 20 column volumes (CV).
6. After identifying the elution volume for the target protein, the slope of the gradient can be leveled out in order to increase the resolution. It is also possible to employ a stepwise gradient.

Pack size

25 mL

BioProcess resin

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Yes
Maximum flow velocity
4.0 mL/min (1 mL), 20 mL/min (5 mL)
Dimensions
0.7 × 2.5 cm (1 mL), 1.6 × 2.5 cm (5 mL)
Column volume
1 mL and 5 mL
Column hardware pressure limit
5 bar (0.5 MPa, 73 psi)

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