Octyl Sepharose 4 Fast Flow

Product Information

Cat#No#	Oc-421C

Product Overview

Octyl Sepharose 4 Fast Flow is a well established, standard aliphatic hydrophobic interaction chromatography (HIC) resin for capture and intermediate purification of larger proteins.
Aliphatic HIC resins based on Sepharose Fast Flow base matrix derivatized via uncharged, chemically-stable ether linkages.
The octyl derivative adds a different and complementary selectivity to the Sepharose Fast Flow HIC product family.
Optimized for the separation of larger proteins in capture and intermediate purification steps.
BioProcess resin supported for industrial applications and well established in approved processes.

Description

Octyl Sepharose 4 Fast Flow is part of the Sepharose Fast Flow HIC platform, which has been an industrial standard for HIC chromatography during recent decades. This resin 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. Octyl Sepharose 4 Fast Flow is available in a range of different bulk pack sizes and convenient pre-packed formats for easy scale-up and process development.

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 20ºC 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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CREATIVE BIOMART®
Chromatography

Octyl Sepharose 4 Fast Flow

Matrix
cross-linked agarose, 4%, spherical
Particle Size
45 µm-165 µm
Average particle size
~90 µm
Ligand
Octyl
Ligand density
~5 µmol Octyl/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.
pH working range
3–13
CIP stability
2–14
Temperature stability
4°C to 40°C
Autoclavable
20 min at 121°C in distilled water pH 7, 5 cycles.
Storage

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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.

Elution

Reducing the concentration of salting-out ions in the buffer with a decreasing salt gradient.
Increasing the concentration of chaotropic ions in the buffer with an increasing gradient.
Eluting with a polarity-reducing organic solvent 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.
To prevent a slow build up of contaminants on the column over time, it is possible that more rigorous cleaning protocols have to be applied on a regular basis.

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Cleaning-in-place
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 followed by 3 to 4 bed volumes of water. Apply gradients to avoid air bubble formation when using high concentrations of organic solvents. Remove other contaminants the following method is suggested: Wash the column with 4 bed volumes of 0.5 to 1.0 M NaOH at 40 cm/h, followed by 2 to 3 bed volumes of water. Alternatively, wash the column with 1 to 2 bed volumes of 0.5% nonionic detergent followed by 5 bed volumes of 70% ethanol to remove the detergent, and 3 to 4 bed volumes of water.
Sanitization
Wash the column with 0.5 to 1.0 M NaOH at a flow velocity of approximately 40 cm/h, contact time 30 to 60 minutes.
Sterilization
To sterilize Butyl Sepharose 4 Fast Flow or Octyl Sepharose 4 Fast Flow, dismantle the column and autoclave the resin for 20 minutes at 121°C.
Pack size
200 mL
BioProcess resin
Yes
Dimensions
5 cm

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