CREATIVE BIOMART®
Chromatography

# Octyl Sepharose CL-4B

## Product Information

**Cat#No#** Oc-422C

**Product Overview**

Octyl-Sepharose CL-4B is a well-proven resin for hydrophobic interaction chromatography (HIC). Octyl immobilized on cross-linked, 4 % agarose

**Description**

Octyl Sepharose CL-4B is a separation resin for hydrophobic interaction chromatography (HIC). Substances are separated on the basis of their different hydrophobicity.
The octyl group is covalently coupled to a cross-linked 4% agarose matrix by ether linkage, giving a hydrophobic resin with minimal leakage and no ionic properties.

**Maximum operating pressure**

150 cm/h at 25 °C, HR 16/10 column, 5 cm bed height.

**Matrix**

cross-linked agarose, 4%, spherical

**Particle Size**

45 µm-165 µm

**Average particle size**

~90 µm

**Ligand**

Octyl

**Ligand density**

40 µmole/mL drained medium

**Recommended column height**

5 cm

Tel: 1-631-559-9269 1-516-512-3133
Email:info@creative-biomart.com
Fax:1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

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| **Chemical stability** |
| --- |
| Stable in commonly used aqueous buffers for HIC chromatography. |
| **Physical stability** |
| Negligible volume variation due to changes in pH or ionic strength. |
| **pH working range** |
| 3–12 |
| **CIP stability** |
| 2–14 |
| **Autoclavable** |
| 121°C for 30 min |
| **Storage** |
| 4 to 30°C, 20% Ethanol or 0.1 M NaOH |
| **Elution buffer** |
| 0.05 M phosphate buffer, pH 7.0. |
| **Binding** |
| The binding of proteins to hydrophobic resins is influenced by:<br>The structure of the ligand, such as a carbon chain or an aromatic ligand. A phenyl group is, for example, less hydrophobic than an octyl group.<br>The concentration and salting-out effect of the binding buffer.<br>Those salts which cause salting-out, for example ammonium sulphate, also promote binding to hydrophobic ligands. The sample is applied in a solution of high concentration of salt. A salt concentration between 0.5 and 2.0 M ammonium sulphate is commonly used1 . The column must be equilibrated at the same concentration.<br>Temperature. Hydrophobic interactions usually decrease with decreasing temperature. |
| **Equilibration** |
| Before applying the sample, equilibrate the column with at least 2 column volumes at chosen binding |

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conditions until the baseline is stable.
A common binding buffer for hydrophobic interaction chromatography is 0.05 M phosphate buffer, 1.7 M $(NH_4)_2SO_4$, pH 7.0.

**Elution**

Reducing the concentration of salting-out ions in the buffer with a decreasing salt gradient.
Eluting with a nonpolar organic solvent.
Including detergent in the eluent.

**Regeneration**

Depending on the nature of the sample, regeneration is normally performed by washing with 2 to 3 bed volumes of an aqueous solution of 30% isopropanol and with 3 bed volumes of distilled water, followed by re-equilibrating in start buffer.
In some applications, substances such as denaturated proteins or lipids do not elute in this regeneration procedure. These can be removed by cleaning-in-place procedures.

**Cleaning-in-place**

Remove precipitated proteins, tightly bound proteins, lipids and lipoproteins by washing the column, in reversed flow direction, with 2 to 3 bed volumes of 0.01 M NaOH. Stop the flow and let it stand for a maximum of 4 hours.
Wash with distilled water, at least 2 to 3 bed volumes, until the pH of the effluent is neutral. Re-equilibrate with at least 3 bed volumes of binding buffer.
Alternatively, wash the column with 4 bed volumes of up to 70% ethanol or 30% isopropanol or with 2 bed volumes of detergent in a basic or acidic solution. Wash at a low flow velocity of approximately 40 cm/h, contact time 1 to 2 hours, reversed flow direction. When using high concentrations of organic solvents, apply increasing gradients to avoid air bubble formation.

**Sanitization**

0.5–1 M NaOH for at least 5 hours.

**Pack size**

200 mL

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