Phenyl Sepharose™ CL-4B

Phenyl Sepharose CL-4B is a separation resin for hydrophobic interaction chromatography (HIC). Substances are separated on the basis of their different hydrophobicity.

The phenyl 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.

Phenyl Sepharose CL-4B belongs to the GE range of BioProcess resins. BioProcess chromatography resins are developed and supported for production scale chromatography. BioProcess resins are produced with validated methods and are tested to meet manufacturing requirements. Secure ordering and delivery routines give a reliable supply of resins for production scale. Regulatory Support Files (RSF) are available to assist process validation and submissions to regulatory authorities. BioProcess resins cover all purification steps from capture to polishing.

Table of Contents

Read these instructions carefully before using the products.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

1 Characteristics

Table 1. Characteristics of Phenyl Sepharose CL-4B

Matrix	Cross-linked agarose, 4%, spherical
Particle size, d_{50V} [1]	~ 90 µm
Ligand concentration	~ 29 µmol/mL resin
Total binding capacity [2]	3 to 5 mg β-lactoglobulin/mL resin
Reccommended maximum operating flow velocity	150 cm/h [3, 4]
pH stability, operational [5]	3 to 12
pH stability, CIP [6]	3 to 12
Chemical stability	Stable to commonly used aqueous buffers
Physical stability	Negligible volume variation due to changes in pH or ionic strength.
Storage	20% ethanol 4°C to 30°C

1 Median particle size of the cumulative volume distribution.

2 Protein in excess is loaded in 0.01 M Phosphate buffer at pH 7 containing 4 M NaCL on a XK 16/20 column. The binding capacity is obtained by measuring the amount of bound and eluted protein in 0.01 M phosphate buffer at pH 7 containing 50% ethylene glycol.

3 In a column with 5 cm diameter and 10 cm bed height using 0.9% sodium chloride solution at room temperature.

4 Flow velocity measured on the base matrix.

5 pH range where resin can be operated without significant change in function.

6 pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

2 Preparing the resin

Phenyl Sepharose CL-4B is supplied preswollen in 20°C ethanol.

General packing procedures

The following parameters refer to large-scale packing:

Preferred packing solution	10% to 20% ethanol or water
Resin slurry concentration	50%
Packing pressure	0.25 bar
Packing flow velocity	60 to 100 cm/h

3 Packing Sepharose CL-4B

1 Equilibrate all material to the temperature at which the chromatography will be performed.

2 Degas the resin slurry.

3 Eliminate air from the column dead spaces by flushing end pieces with ethanol or water. Make sure no air has been trapped under the column net. Close the column outlet with a few centimeters of ethanol or water remaining in the column.

4 Pour the slurry into the column in one continuous motion. Pouring the slurry down a glass rod held against the wall of the column will minimize the introduction of air bubbles.

5 Immediately fill the remainder of the column with ethanol or water, assemble the column top piece onto the column and connect the column to a pump.

6 Open the bottom outlet of the column and set the run at the desired flow rate. The general recommendation is to use a packing flow velocity between 60 to 100 cm/h. Observe that the packing flow velocity also depends on the bed height to be packed.

Note: *If you have packed at the maximum flow velocity, do not exceed 75% of this in subsequent chromatographic procedures.*

7 Maintain the packing flow rate for 3 bed volumes after constant bed height is reached.

4 Using an adapter

1. After the resin has been packed as described above, close the column outlet and remove the top piece from the column. Carefully fill the rest of the column with ethanol or water to form an upward meniscus at the top.
2. Insert the adapter at an angle into the column, ensuring that no air is trapped under the net.
3. Make all tubing connections at this stage. There must be a bubble-free liquid connection between the column and the pump, and column and the sample application system.
4. Slide the plunger slowly down the column so that the air above the net and in the capillary tubings is displaced by eluent. Valves on the inlet side of the column must be turned in all directions during this procedure to make sure that air is removed.
5. Lock the adapter in position on the resin surface, open the column outlet and start the eluent flow. Pass eluent through the column at the packing flow rate until the resin bed is stable. Re-position the adapter on the resin surface as necessary.

5 Equilibration

Before applying the sample, equilibrate the column with at least 2 column volumes at chosen binding 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.

6 Binding

The binding of proteins to the hydrophobic resin is influenced by:

- 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.
- The concentration and salting out effect of the binding buffer (see the Hofmeister series in Table 2).
- 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 used [1]. The column must be equilibrated at the same concentration.
- Temperature. Hydrophobic interactions usually decrease with decreasing temperature.

[1] When working with proteins which have a tendency to aggregate, start with a lower $(NH_4)_2SO_4$ concentration to avoid the risk of precipitation.

7 Elution

Bound proteins are eluted by reducing 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)
- eluting with a nonpolar organic solvent
- including detergent in the eluent

Table 2. The Hofmeister series. Increasing the salting-out effect strengthens the hydrophobic interactions, whereas increasing the chaotropic effect weakens them.

← Increasing salting-out effect

Anions:	PO_4^{3-}	SO_4^{2-}	CH_{3COO}^-	Cl^-	Br^-	NO_3^-	ClO_4^-	I^-	SCN^-
Cations:		NH_4^+	Rb^+	K^+	Na^+	Cs^+	Li^+	MG^{2+}	Ba^{2+}

Increasing chaotropic effect →

As a start, we suggest a linear gradient from 0 to 100% Elution buffer with: Start buffer (A): 0.05 M phosphate buffer, pH 7.0 + 1.7 M $(NH_4)_2SO_4$ Elution buffer (B): 0.05 M phosphate buffer, pH 7.0.

8 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 denatured proteins or lipids do not elute in this regeneration procedure. These can be removed by cleaning-in-place procedures.

9 Cleaning-in-place (CIP)

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.

Detergents are difficult to remove and are recommended only if the other procedures fail to give satisfactory results. After treatment with detergent, wash with 5 bed volumes of 70% ethanol, or more, to remove residual detergents.

Re-equilibrate the column with at least 3 bed volumes of binding buffer.

CAUTION

70% ethanol can require the use of explosion-proof areas and equipment.

10 Sanitization

Sanitization reduces microbial contamination of the resin bed to a very low level.

Wash 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 sterile start buffer.

Column performance is normally not significantly changed by the cleaning-in-place or sanitization procedures described above.

11 Storage

Store the resin in 20% ethanol at 4°C to 30°C.

12 Ordering information

Product	Quantity	Product code
Phenyl Sepharose CL-4B	10 L	17081005
Phenyl Sepharose CL-4B	200 mL	17081001

Related products	Quantity	Product code
Phenyl Sepharose 6 Fast Flow (high sub)	25 mL	17097310
Phenyl Sepharose 6 Fast Flow (high sub)	200 mL	17097305
Phenyl Sepharose 6 Fast Flow (low sub)	25 mL	17096510
Phenyl Sepharose 6 Fast Flow (low sub)	200 mL	17096505
Phenyl Sepharose High Performance	75 mL	17108201
Hiprep 16/10 FF	1	17509501
Hiprep 16/10 FF	1	17509401
HiTrap Phenyl FF (high sub)	5 x 1 mL	17135501
HiTrap Phenyl FF (high sub)	5 x 5 mL	17519301
HiTrap Phenyl FF (low sub)	5 x 1 mL	17135301
HiTrap Phenyl FF (low sub)	5 x 5 mL	17519401
HiTrap Phenyl HP	5 x 1 mL	17135101
HiTrap Phenyl HP	5 x 5 mL	17519501
HiTrap HIC Selection Kit	7 x 1 mL	28411007

Handbook	Product code
Hydrophobic Interaction and Reversed Phase Chromatography	11001269

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