CREATIVE BIOMART®
Chromatography

Phenyl Sepharose High Performance

Product Information

Cat#No# Ph-426C

Product Overview

Phenyl Sepharose High Performance is an aromatic hydrophobic interaction chromatography (HIC) medium, designed for intermediate and polishing step purification steps when high resolution has priority.
Aromatic HIC resin designed for intermediate purification and polishing steps.
Selectivity similar to Phenyl Sepharose 6 Fast Flow (low sub) but higher resolution due to the smaller 34 µm bead size.
BioProcess resin supported for industrial applications and well-established in approved processes.
The hydrophilic nature of the base matrix ensures low levels of non-specific binding.

Description

Phenyl Sepharose High Performance and Butyl Sepharose High Performance are hydrophobic interaction chromatography (HIC) resins.

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.

Packing Column

Preferred packing solution: 10% to 20% ethanol.
Resin slurry concentration: 50%.
Packing pressure: 0.3 to 0.6 MPa (3 to 6 bar, 43.6 to 87.0 psi).
Packing flow velocity: 200 to 300 cm/h.

Matrix

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Phenyl Sepharose High Performance

cross-linked agarose, spherical
Average particle size
~34 µm
Ligand
Phenyl
Ligand density
Approx. 25 µmol phenyl/ml gel
Recommended flow rate
≤ 100 cm/h
Recommended column height
30 cm
Chemical stability
Stable in commonly used aqueous buffers - 1.0 M NaOH, 1 M Acetic acid, 30% Acetonitrile, 70% Ethanol, 30% Isopropanol, 6 M guanidine-hydrochloride, 8 M Urea, 3 M ammoniumsulfate, ionic detergents, non-ionic detergents, polar organic solvents, 2% sodium dodectyl sulfate.
pH working range
3–13
CIP stability
2–14
Storage
4 to 30°C, 20% Ethanol
Evaluation of Packing
Test column efficiency to check the quality of the packing. Tests must be made directly after packing and at regular intervals during the working life of the column plus when separation performance is seen to deteriorate.

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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.
To prevent a slow buildup of contaminants on the column over time, you maybe have to apply more rigorous cleaning protocols on a regular basis.

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.

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

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