

Phenyl Sepharose 6 Fast Flow (Low Sub)

Product Information

Cat#No# Ph-424C

Product Overview

Phenyl Sepharose 6 Fast Flow is a well established, standard aromatic hydrophobic interaction chromatography (HIC) resin for capture and intermediate purification requiring low to resin hydrophobicity. Standard aromatic HIC resins based on Sepharose Fast Flow base matrix derivatized via uncharged, chemically-stable ether linkages. Designed for initial and intermediate purification requiring low to medium hydrophobicity.

Two levels of substitution help find optimal selectivity and binding capacity for a given application. 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 6 Fast Flow is part of the Sepharose Fast Flow HIC platform, which has been an industrial standard for HIC chromatography during recent decades. Phenyl Sepharose 6 Fast Flow is composed of cross-linked, 6% agarose beads modified with standard aromatic phenyl groups via uncharged, chemically-stable ether linkages. Two levels of ligand substitution degree help to find the optimal selectivity and binding capacity for a given application.

Characteristic

High dynamic binding capacity and stability.

Fast Flow matrix gives high flow rates.

Highly hydrophilic base matrix making true hydrophobic interaction chromatography possible without interfering secondary interactions influencing protein conformation or binding.

Suitable for a wide range of applications from research to production scale.

Maximum operating pressure

250-400 cm/h at <0.1 MPa in a XK 50/60 column with 5 cm diameter and 25 cm bed height (at 20°C using buffers with the same viscosity as water).

Matrix

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cross-linked agarose, 6%, spherical

Particle Size

45 µm-165 µm

Average particle size

~90 µm 2022/5/16~90 µm

Ligand

Phenyl

Ligand density

~25 µmol phenyl/mL resin

Recommended column height

25 cm

Chemical stability

Stable in commonly used aqueous buffers - 1.0 M NaOH, 3 M Ammonium sulphate, 30% isopropanol, 70% ethanol, 10% ethylene glycol, 0.5% SDS, 6 M guanidine-hydrochloride, 8 M Urea.

pH working range

3–13

CIP stability

2–14

Temperature stability

4°C to 30°C

Autoclavable

20 min at 121°C in distilled water pH 7, 5 cycles.

Storage

4 to 30°C, 20% Ethanol

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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 (A_s). These values are easily determined by applying a sample such as 1% acetone solution to the column. Sodium chloride can also be used as a test substance. Use a concentration of 0.8 M NaCl in water as sample and 0.4 M NaCl in water as eluent.

Binding

High ligand concentration does not necessarily correspond to high capacity regarding adsorption of protein, but a high ligand concentration can encourage multipoint attachment of proteins which otherwise might have difficulty adsorbing to lower ligand concentrations.

A moderate ligand concentration enables the possibility to selectively bind the protein of interest by adjustment of the binding buffer concentration.

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 ionic strength. A salt concentration just below that employed for salting out the protein is standard, usually 1.7 M $(\text{NH}_4)_2\text{SO}_4$. The column must be equilibrated at the same ionic strength.

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 in a positive gradient.

Eluting with a polarity-reducing organic solvent.

Including detergent in the eluent.

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 precipitated proteins: Wash the column with 4 bed volumes of 0.5 to 1.0 M NaOH solution at a flow velocity of 40 cm/h, followed by 2 to 3 bed volumes of water.

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. Alternatively, wash the column with detergent in a basic or acidic solution. Wash at a flow velocity of 40 cm/h. Residual detergent can be removed by washing with 5 bed volumes of 70% ethanol.

Sanitization

For inactivation of microbial contaminants, equilibrate 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.

Wash the column thoroughly with running buffer after sanitization.

Sterilization

To sterilize Phenyl Sepharose 6 Fast Flow (low sub and high sub), dismantle the column and autoclave the resin for 20 minutes at 121°C.

Pack size

25 mL

BioProcess resin

Yes

Dimensions

5 cm
