Phenyl Sepharose™ 6 Fast Flow (low sub and high sub)

Phenyl Sepharose 6 Fast Flow (low sub and high sub) are part of the GE resin range for hydrophobic interaction chromatography (HIC). They are also part of the Fast Flow resin range developed for preparative separations. They are based on rigid, ~ 90 µm diameter cross-linked, 6% agarose particles, designed to meet industrial demands on reliability and scalability.

These instructions contain information about resin characteristics, column packing, and maintenance. For best performance and trouble-free operation, read these instructions before using Phenyl Sepharose 6 Fast Flow (low sub and high sub).

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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 Introduction

BioProcess™ resins

Phenyl Sepharose 6 Fast Flow (low sub and high sub) belong to the 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.

Description

The base matrix, Sepharose 6 Fast Flow, is a cross-linked, 6% agarose derivative with excellent kinetics, making them ideal for process scale applications, particularly during initial capture and intermediate stages of a separation process, when high flow rates are required.

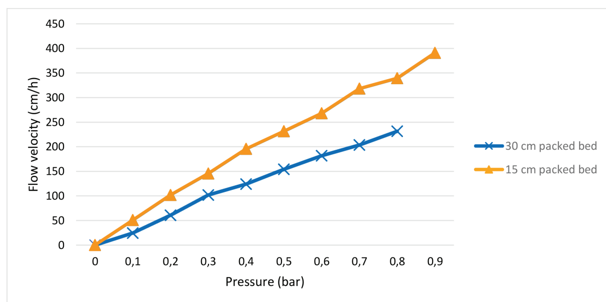


Fig 1. Typical pressure/flow curves for Phenyl Sepharose 6 Fast Flow High sub/Low sub, packed bed in a BPG 300 column with bed heights 15 cm and 30 cm.

The high physical and chemical stability of the matrix prevents bed compression and formation of fines, and allows efficient maintenance procedures for increased resin life time. Typical pressure/flow curves are shown in Fig 1.

Method design and optimization

The main purpose of optimizing a chromatographic step is to reach the predefined purity level with highest possible product recovery by choosing the most suitable combination of the critical chromatographic parameters. In process chromatography, in contrast to analytical or small scale preparative chromatography, this has to be accomplished as quickly and economically as possible. That means finding the conditions that give the highest possible productivity and process economy.

Recommendations for optimizing the critical operational parameters which affect the maximum utilization of a HIC step can be found in our handbook: *Hydrophobic Interaction and Reversed Phase Chromatography: Principles and Methods*, (11001269), available from your local GE office.

Resin screening

Table 3 shows the kits that help with screening and selection of resin.

Screening kit	Description
PreDictor™ HIC Screening High Hydrophobicity	Phenyl Sepharose 6 Fast Flow (low sub) Capto™ Butyl Phenyl Sepharose Fast Flow (high sub) Capto Phenyl (high sub)
PreDictor HIC Screening Low Low Hydrophobicity	Butyl-S Sepharose 6 Fast Flow Octyl Sepharose 4 Fast Flow Butyl Sepharose 4 Fast Flow Capto Octyl
HiTrap™ HIC Test Kit 7×1 mL columns	Phenyl Sepharose 6 Fast Flow (high sub) Phenyl Sepharose 6 Fast Flow (low sub) Phenyl Sepharose High Performance Butyl Sepharose 4 Fast Flow Butyl-S Sepharose 6 Fast Flow Octyl Sepharose 4 Fast Flow

Characteristics of Phenyl Sepharose 6 Fast Flow (low sub and high sub)

The following table shows the characteristics of Phenyl Sepharose 6 Fast Flow (low sub and high sub).

Matrix	Cross-linked agarose, 6% , spherical
Type of ligand	Phenyl: $R-O-CH_2-CH(OH)-CH_2-O-C_6H_5$
Particle form	Rigid, spherical, macro porous
Particle size, d_{50V} [1]	~ 90 µm
Ligand concentration	~ 25µmol phenyl/mL resin (low sub) ~ 45µmol phenyl/mL resin (high sub)
Pressure/flow characteristics	250 to 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) [2] [3]
pH stability, operational [4] **pH stability, CIP** [5]	3 to 13 2 to 14
Operating temperature	4°C to 30°C
Chemical stability	Stable to commonly used aqueous buffers, 1.0 M NaOH [6], 3 M ammonium sulphate [7], 70% ethanol, 30% isopropanol, 0.5% SDS, 6 M guanidine hydrochloride, 8 M urea, 10% ethylene glycol
Autoclavability	20 min at 121°C in distilled water pH 7 for 5 cycles
Delivery conditions	20% ethanol
Storage	20% ethanol, 4°C to 30°C Do not freeze

1 Median particle size of the cumulative volume distribution.

2 The pressure/flow characteristics describes the relationship between pressure and flow under the set circumstances. The pressure given shall not be taken as the maximum pressure of the resin.

3 Pressure/flow test performed on the base matrix.

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

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

[6] 1.0 M NaOH must only be used for cleaning purposes.

[7] Due to instability, ammonium sulphate is not suitable when working at pH values above 8.0.

2 General column packing guidelines

Recommended columns

This section lists columns that are recommended by GE.

Lab scale:

- Tricorn™, i.d. 5 to 10 mm, bed volumes up to 24 mL, bed heights up to 30 cm
- HiScale™, i.d. 16 to 50 mm, bed volumes up to 785 mL, bed heights up to 40 cm
- XK, i.d. 16 to 50 mm, bed volumes up to 559 mL, bed heights up to 28 cm

Production scale:

- BPG, i.d. 100 to 450 mm, bed volumes up to 130 L, bed heights up to 58 cm
- Chromaflow™, i.d. 280 to 2000 mm, bed volumes up to 1570 L, bed heights up to 50 cm
- AxiChrom™, i.d. 50 to 200 mm, bed volumes up to 16.7 L, bed heights up to 50 cm
- AxiChrom, i.d. 300 to 1600 mm, bed volumes up to 1005 L, bed heights up to 50 cm

General considerations

Purifying biological macromolecules by HIC is a typical high selectivity technique where the difference in retention for the molecules to be separated can be substantial at any specific ionic strength.

Therefore, relatively short columns can be used if the selectivity of the adsorbent is exploited in an optimal way. Typical bed heights range from 3 to 15 cm, which will minimise back pressure and allow high throughput.

Columns can be packed in different ways depending on the type of column and equipment used. Always read and follow the relevant column instruction manual carefully. Sepharose 6 Fast Flow based resins are easy to pack since their rigidity allows the use of high flow rates.

Two types of packing methods are described:

- Pressure packing (for columns with adapters)
- Chromaflow packing

The following parameters refers to large-scale packing:

General packing procedures	
Preferred packing solution	10% to 20% ethanol
Resin slurry concentration	50%
Packing pressure	1.3 to 1.5 bar
Packing flow velocity	400 to 600 cm/h

How well the column is packed will have a major effect on the result of the separation. It is therefore very important to pack and test the column according to the following recommendations.

Begin the packing procedure by determining the optimal packing flow rate. Guidelines are given below for determining the optimal packing flow rates for columns with adapters and fixed bed heights.

Determining optimal packing flow rates

The optimal packing flow rate is dependent on column size and type, resin volume, packing solution, and temperature. The optimal packing flow rate must therefore be determined empirically for each individual system.

To determine the optimal packing flow rate, follow these instructions:

Step	Action
1	Calculate the amount of resin needed for the slurry (this is especially important for columns with fixed bed heights). The quantity of resin required per liter packed volume is approximately 1.15 L sedimented resin.
2	Prepare the column exactly as for column packing.
3	Begin packing the resin at a low flow velocity (30 cm/h).
4	Increase the pressure in increments and record the flow rate when the pressure has stabilised. Do not exceed the maximum pressure of the column, or the maximum flow rate for the resin.

Step	Action
5	The maximum flow rate is reached when the pressure/flow curve levels off or the maximum pressure of the column is reached. Stop the packing and do not exceed this flow rate. The optimal packing flow rate/pressure is 70% to 100% of the maximum flow rate/pressure.
6	Plot the pressure/flow curve as in *Description, on page 3* and determine the optimal packing flow rate.

The operational flow rate/pressure must be < 70% of the packing flow rate/pressure.

3 Packing XK columns

Introduction

The following instructions are for packing XK columns, but can be modified for columns of different dimensions by maintaining the packing pressure at 2 bar (0.2 MPa).

Equipment needed

To pack a XK column, you need the resin, a column, a second column that will act as a reservoir for the resin slurry, the top adapter, the bottom end piece, a packing connector to join the column and the reservoir, stop plugs, a syringe connector, pump, and general lab equipment.

Preparation of the resin

Phenyl Sepharose 6 Fast Flow (low sub and high sub) are supplied preswollen in 20% ethanol with a slurry concentration of 75%. For best results, a slurry concentration of 50% in distilled water is recommended.

Packing with Sepharose 6 Fast Flow

To prepare a XK column for packing, follow these instructions.

Step	Action
1	Equilibrate all material to the temperature at which the chromatography will be performed.
2	De-gas the resin slurry.
3	Remove all traces of air in the bottom end piece by forcing 20% ethanol up through the net using a syringe connected to the tubing. Attach the stop plug, insert the end piece into the column and tighten securely. Next, add a height of about 2 centimeters of distilled water to the bottom of the column.
4	Attach the packing connector to the column making sure it fits snugly. Attach the reservoir to the packing connector, again, tightening securely. Level the column.

To pack a XK column with Sepharose 6 Fast Flow, follow these instructions.

Step	Action
1	Begin packing the column by reslurrying the resin to make sure it becomes homogene. Pour the resin through the reservoir into the column at once, in one single motion, by decanting down a spatula. This gives an even presentation of resin slurry and prevents formation of air bubbles.
2	Eliminate air from the top adapter as before, by forcing 20% ethanol through the adapter with a syringe connected to the top. Insert the adapter to meet the resin slurry and fasten in place. If your packing configuration does not include an adapter, fill liquid to the top of the reservoir. Making a drop-to-drop connection, attach the pump to the column.
3	Ideally, Sepharose 6 Fast Flow is packed at a constant pressure 2 bar (0.2 MPa) in XK columns. If the packing equipment does not include a pressure gauge, use a packing flow rate of 20 mL/min (XK 16), 35 mL/min (XK 26), 110 mL/min (XK 50). ***Note:*** *Do not exceed 75% of the packing flow rate in subsequent chromatographic procedures. Remove the bottom stop plug and start the pump.*
4	Maintain the packing flow rate for 3 bed volumes after a constant bed height is reached.
5	Mark the level of the resin in the column with a marking pen. Then stop the pump and stopper the column. Over a beaker or a sink, detach the reservoir from the column.

To reassemble a XK column after packing, follow these instructions.

Step	Action
1	Assemble the column and fill the space above the resin with distilled water. Remove air from the adapter as before and place it at a 45° angle into the top of the column. Gently lower the adapter.

Step	Action
2	Using a pipette, remove any resin particles and liquid from the upper portion of the column that can lie in the thread path of the adapter.
3	Lower the ring and tighten the adapter. Now slide the adapter down to meet the packed resin, expelling liquid out through the top as the adapter is pressed down.
4	Press the adapter approximately three millimeters below the surface of the resin bed and firmly tighten the top adapter.
5	Run the column to check for any leaks or gaps. The packed column is now ready for use.

Binding

The binding of proteins to hydrophobic resins is influenced by:

- the structure of the ligand, for example carbon chain or an aromatic ligand
- the ligand concentration
- the ionic strength of the buffer
- the salting-out effect (see *Table* The Hofmeister series)
- the temperature

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 $(NH_4)_2SO_4$. The column must be equilibrated at the same ionic strength.

The hydrophobic interactions will be weaker if the experiment is done in a cold room.

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)
- increasing the concentration of chaotropic ions in the buffer in a positive gradient (linear or step)
- eluting with a polarity-reducing organic solvent, for example ethylene glycol
- including detergent in the eluent

The Hofmeister series

	← Increasing precipitation ("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 ("salting-in") effect →

Increasing the precipitation effect strengthens the hydrophobic interactions, whereas increasing the chaotropic effect weakens them. It is recommended to start with a linear gradient from 0% to 100% B with:

Buffer A: 50 mM phosphate buffer, pH 7.0 + 1.7 M $(NH_4)_2SO_4$

Note: *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.*

Buffer B: 50 mM phosphate buffer, pH 7.0

4 Packing methods

Packing AxiChrom columns

AxiChrom columns are packed using mechanical axial compression packing. For more information regarding the AxiChrom columns and packing procedures see:

- AxiChrom Columns (28929041)
- AxiChrom 50, 70 and 100 columns, *Operating instructions* (28933108)
- AxiChrom 140 and 200 columns, *Operating instructions* (28943123)
- AxiChrom 300-1600 columns, *Operating instructions* (29065430)

Pressure packing of BPG columns

BPG glass columns are supplied with a movable adapter. They are packed by conventional pressure packing by pumping the packing solution through the chromatographic bed at a constant flow rate (or back pressure).

Step	Action
1	Pour some water (or packing solution) into the column. Make sure that there is no air trapped under the bottom net. Leave about 2 cm of liquid in the column.
2	Mix the packing buffer with the resin to form a 50% to 70% slurry. (Sedimented bed volume/slurry volume = 0.5 to 0.7). Pour the slurry into the column. Insert the adapter and lower it to the surface of the slurry, making sure no air is trapped under the adapter. Secure the adapter in place.
3	Seal the adapter O-ring and lower the adapter a little further into the slurry to fill the adapter inlet with packing solution.
4	Connect a pump and a pressure gauge, then start packing at the predetermined packing flow rate (or pressure). Keep the flow rate (or pressure) constant during packing and check the pressure at the column inlet. Never exceed the pressure limit for column or resin.

Step	Action
5	When the bed has stabilized, mark the bed height on the column tube, close the bottom valve and stop the pump. The bed starts rising in the column. Loosen the O-ring and lower the adapter to 0.5 to 1 cm from the bed surface.
6	Seal the O-ring, start the pump and continue packing. Repeat steps 5 and 6 until there is a maximum of 1 cm between bed surface and adapter when the bed has stabilized.
7	Close the bottom valve, stop the pump, disconnect the column inlet and push the adapter down to approximately 3 mm below the mark on the column tube without loosening the adapter O-ring. The packing solution will flush the adapter inlet. Remove any trapped air by pumping liquid from the bottom (after the inlet tubing and the bottom valve have been properly filled).

Packing Chromaflow columns

Prepare the column for packing as described in the *Operating instructions*.

This section describes how to pack from the top, or from below.

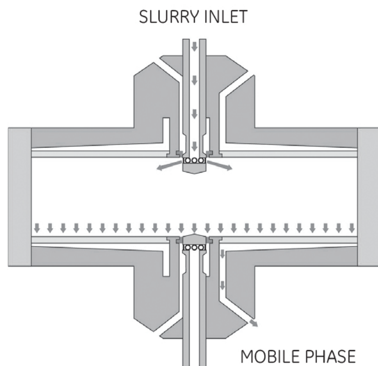
To pack from the top, follow these instructions:

Step	Action
1	Set the top nozzle to the pack position (mid-position).
2	Fully retract the bottom nozzle (run position).
3	Make sure that the top mobile phase is closed.
4	Open the bottom mobile phase.
5	Open Inlet **(C)** and start the packing pump. Adjust the flow to achieve the required packing conditions for the selected resin. Monitor column pressure and the outlet flow rate in order to record column packing parameters. Remember to stir the resin slurry during packing to prevent it from settling.
6	Continue pumping until the column is fully packed and the pump stalls due to buildup of resin in its pipelines. Turn off the packing pump.

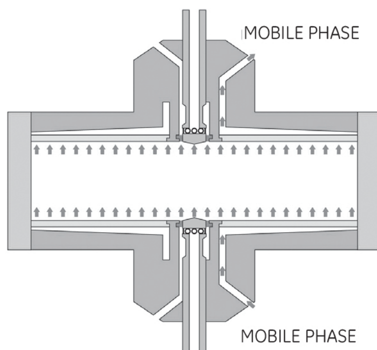
Step	Action
7	Fully retract the top nozzle to its run position. Close Outlet **(C)**. Open Inlet **(B)** from the water/buffer tank and open Outlet **(D)**. The pump must now be restarted to rinse the top slurry lines. If the nozzle is full of liquid when in the packing position, make sure that the waste slurry outlet is open before retracting the nozzle.
8	To clean-in-place, exchange the buffer tank for wash/buffer tank containing cleaning solution.

To pack from below, carry out the same procedure for the connections and flow path via the bottom nozzle. The column is now ready to equilibrate and test.

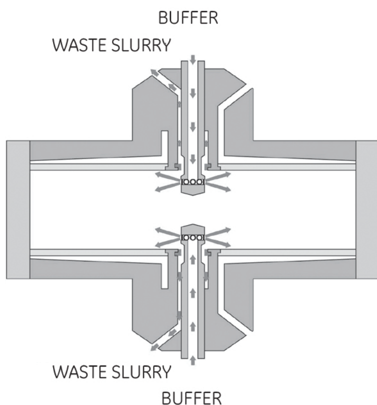
The illustration below shows the principles of operating Chromaflow columns.



Packing position
The top nozzle is extended part of the way (mid position) into the column. The bottom nozzle is fully retracted. Slurry enters the column via the top nozzle and excess liquid exits via the bottom mobile phase outlet. After packing, the slurry lines are isolated from the mobile phase and can be cleaned independently from the rest of the column.



Running position
The bottom and top nozzles are retracted. Mobile phase enters the column directly into an annulus, immediately behind the bed support. The annulus is cut through at an angle to make sure that linear flow rate is kept constant during distribution of the mobile phase across the bed.



Unpacking position
In this position, both bottom and top nozzles are fully extended into the column, thereby exposing a third passage through which resin leaves the column. Cleaning solution can be pumped through the nozzles and sprayed into the column. In this way the column is easily and effectively cleaned without exposing the interior or the resin to the environment, or without dismantling the column.

Note: *It is also possible to use a slightly different packing method where the amount of resin is predetermined. In this case the complete amount of resin is packed into the column causing compression of the bed. When all resin has entered the column the pump is stopped, the top nozzle is retracted, the bottom mobile phase valve closed, and the resin is allowed to decompress within the column.*

5 Evaluation of column packing

Intervals

Test the column efficiency to check the quality of packing. Testing must be done after packing and at regular intervals during the working life of the column and also when separation performance is seen to deteriorate.

Column efficiency testing

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.

For more information about column efficiency testing, consult the application note *Column efficiency testing* (28937207).

Note: *The calculated plate number will vary according to the test conditions and must only be used as a reference value. It is important that test conditions and equipment are kept constant so that results are comparable. Changes of solute, solvent, eluent, sample volume, flow velocity, liquid pathway, temperature, chromatography system, etc. will influence the results.*

Sample volume and flow velocity

For optimal column efficiency results, the sample volume must be approximately 1% of the column volume and the flow velocity 30 cm/h. If an acceptance limit is defined in relation to column performance, the column plate number can be used as one of the acceptance criteria for column use.

Method for measuring HETP and A_s

Calculate HETP and A_S from the UV curve (or conductivity curve) as follows:

$$HETP = \frac{L}{N}$$

L = bed height (cm)
N = number of theoretical plates

$$N = 5.54 \times \left(\frac{V_R}{W_h}\right)^2$$

V_R = volume eluted from the start of sample application to the peak maximum
W_h = peak width measured as the width of the recorded peak at half of the peak height
V_R and W_h are in the same units

The concept of reduced plate height is often used for comparing column performance.

The reduced plate height, h, is calculated as follows:

$$h = \frac{HETP}{d_{50v}}$$

d_{50V} = Median particle size of the cumulative volume distribution (cm)

As a guideline, a value of < 3 is very good.

The peak must be symmetrical, and the asymmetry factor as close to 1 as possible. A typical acceptable range could be $0.8 < A_S < 1.8$.

A change in the shape of the peak is usually the first indication of bed deterioration due to excessive use.

Peak asymmetry factor calculation:

$$A_s = \frac{b}{a}$$

a = ascending part of the peak width at 10% of peak height
b = descending part of the peak width at 10% of peak height

The Figure below shows a UV trace for acetone in a typical test chromatogram from which the HETP and A_S values are calculated.

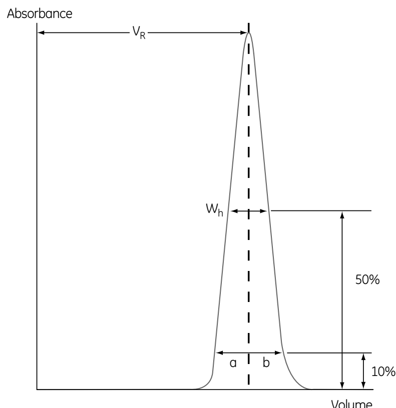


Fig 2. A typical test chromatogram showing the parameters used for HETP and A_S calculations.

6 Maintenance

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.

Cleaning-in-place (CIP)

Cleaning-in-place is the removal of very tightly bound, precipitated or denatured substances from the purification system generated in previous purification cycles. If such contaminants accumulate on the column, they can affect the chromatographic properties of the column. If the fouling is severe, it can also block the column, increasing back pressure and reducing flow rate.

The following are suggested methods to 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.
- 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.

CAUTION

Specific regulations can apply when using 70% ethanol since it can require the use of explosion-proof areas and equipment. Consult your local safety regulations for more information.

To 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.

The CIP protocols given above must be used as guidelines when formulating a cleaning protocol specific for the raw material used. The frequency of CIP will depend on the raw material applied to the column, but it is recommended to use a CIP procedure at least every 5 cycles during normal use. Depending on the nature of the contaminants, it can be necessary to use a combination of different protocols. If fouling is severe, it can be necessary to further optimize the protocols. During CIP flow direction through the column must be reversed.

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.

Storage

Store Phenyl Sepharose 6 Fast Flow (low sub and high sub) in 20% ethanol at 4°C to 30°C, to avoid microbiological growth.

7 Ordering information

This chapter lists product codes for products, handbooks, and data files that relate to Phenyl Sepharose 6 Fast Flow (low sub and high sub). For further information, see www.gelifesciences.com

Product	Quantity	Product code
Phenyl Sepharose 6 Fast Flow (low sub)	25 mL	17096510
	200 mL	17096505
	1 L	17096503
	5 L	17096504
Phenyl Sepharose 6 Fast Flow (high sub)	25 mL	17097310
	200 mL	17097305
	1 L	17097303
	5 L	17097304
	10 L	17097306
	60 L [1]	17097360
HiTrap HIC Test Kit	7×1 mL	28411007
HiTrap Phenyl FF (Low Sub)	5×1 mL	17135301
	5×5 mL	17519401
HiTrap Phenyl FF (High Sub)	5×1 mL	17135501
	5×5 mL	17519301
PreDictor Screening High Hydrophobicity	6 µL	28992392
	50 µL	28992397
PreDictor Screening Low Hydrophobicity	6 µL	28992395
	50 µL	28992398

[1] Pack sizes available upon request

Handbook	Product code.
Hydrophobic Interaction and Reversed Phase Chromatography: Principles and Methods	11001269

The complete range of Sepharose 6 Fast Flow resins includes other HIC resins as well as resins for ion exchange and affinity chromatography.

Data File	Product code.
BPG 100, 140, 200, 300 and 450 series	18111523
Chromaflow columns	18113892
AxiChrom Columns	28929041
HiTrap HIC Selection Kit	18114321
Phenyl Sepharose 6 Fast Flow (low sub and high sub)	18102053

Instructions	Product code
Predictable scale-up through column design and robust packing methodology	28949052
Constant Flow Packing method	29001795
Pack-in-place packing procedure	29001797
AxiChrom 50, 70 and 100 columns, *Operating instructions*	28933108
AxiChrom 140 and 200 columns, *Operating instructions*	28943123
AxiChrom 300-1600 columns, *Operating instructions*	29065430
HiTrap HIC Columns	11003452

Selection Guide	Product code
Hydrophobic Interaction Chromatography Selection Guide	29022223

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