Phenyl Sepharose™ 6 FastFlow (low sub)
Phenyl Sepharose 6 FastFlow (high sub)

Phenyl Sepharose 6 Fast Flow (low substitution) and Phenyl Sepharose 6 Fast Flow (high substitution) are part of the GE Healthcare Life Sciences range of media for hydrophobic interaction chromatography (HIC) (Fig 1). Both media satisfy process chromatography requirements in terms of performance, stability, scaleability, and bulk availability. As part of the BioProcess™ range of media, Phenyl Sepharose 6 Fast Flow (low sub) and Phenyl Sepharose 6 Fast Flow (high sub) carry comprehensive technical and regulatory support for production scale applications. The media have long track records of use in downstream processing of biopharmaceuticals.

- 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

Fig 1. Phenyl Sepharose 6 Fast Flow (low sub) and Phenyl Sepharose 6 Fast Flow (high sub) for rapid purification.

HIC technology

HIC is widely used for purification of macromolecules (proteins and peptides). Substances are separated on the basis of their varying strength of hydrophobic interaction with hydrophobic groups attached to an uncharged gel matrix. This technique is usually performed in the presence of moderately high concentrations of anti-chaotropic salts (salt-promoted adsorption chromatography).

Several factors influence the chromatographic behaviour of proteins and peptides on hydrophobic media. Some of these factors are crucial for developing an optimized purification procedure. Purification protocols for analytical or preparative separations in small scale, with emphasis on resolution, are different from those in a manufacturing process where the emphasis is on the highest possible productivity.

Parameters that influence binding, resolution, selectivity, and recovery include:

- Ligand structure (aliphatic or aromatic)
- Ligand density
- Sample characteristics
- Flow rate
- Salting-out effect
- Ionic strength
- Temperature
- pH

The dynamic binding capacity of HIC media, which is affected by the type of buffer ion used, decreases with increasing flow rate.

Resolution is also affected by the substitution level. For adsorption of proteins, high ligand density does not necessarily correspond to high capacity, but can encourage multipoint attachment of proteins that otherwise might not adsorb to a medium with a lower degree of substitution.

A moderate ligand density can enable the user to adjust the binding buffer concentration and bind the protein of interest selectively. During desorption, parameters such as buffer composition, gradient volume, flow rate, and gradient shape (linear or step-wise) play an important role.

HIC is compatible with other chromatographic techniques commonly used in purification schemes. For example, after ammonium sulphate precipitation, ion exchange, and affinity chromatography, the sample is often left in a high salt concentration. With HIC as the next step, it is usually possible to transfer the sample directly, eliminating the need for prior dialysis or gel filtration. As elution of the protein of interest from a HIC column often leaves the substance at low ionic strength, HIC is a practical step before techniques such as gel filtration, ion exchange, reversed phase, and affinity chromatography.

Phenyl Sepharose 6 Fast Flow

Phenyl Sepharose 6 Fast Flow (low sub) and Phenyl Sepharose 6 Fast Flow (high sub) are based on highly cross-linked 6% agarose. Sepharose 6 fast Flow is a rigid matrix that enables rapid processing of large volumes. Both media are suitable for applications at all scales from laboratory to production.

At process scale, GE Healthcare Fast Flow HIC media are particularly useful during the initial and intermediate stages of a separation process when high flow rates are required. At production scale, the media meet the requirements for reliable and economic purification of biological material. Phenyl Sepaharose 6 Fast Flow (high sub) and Phenyl Sepaharose 6 Fast Flow (low sub) were originally developed and tested in cooperation with leading large-scale manufacturers and are used today in routine commercial pharmaceutical production. For information on additional GE Healthcare HIC chromatography media suitable for large-scale manufacturing, please visit www.gelifesciences.com/capto.

Stability

Phenyl Sepharose 6 Fast Flow (low sub) and Phenyl Sepharose 6 Fast Flow (high sub) are compatible with commonly used aqueous buffers. The media have high chemical and mechanical stability and withstand high concentrations of denaturing agents such as urea and guanidine hydrochloride. Both media have high thermal stability and are autoclavable at 121°C for 20 minutes. Table 1 summarizes the medium characteristics.

Production-scale use

Columns

A list of columns recommended for Phenyl Sepharose 6 Fast Flow (low sub) and Phenyl Sepharose 6 Fast Flow (high sub) media in semipreparative and process scale operations can be found in Table 2. The working flow rate should not exceed 80% of the packing flow rate.

Figure 2 shows pressure/flow curves for the media packed in an XK 50130 Column.

Table 2. Recommended columns for Phenyl Sepharose 6 Fast Flow (low sub) and Phenyl Sepharose 6 Fast Flow (high sub)

Column	Recommended bed height	Bed volume
XK 50/20 Column	10 cm	0.2 L
BioProcess BPG 100/500 glass column	10 cm	0.8 L
BioProcess BPG 200/500 class column	10 cm	3.2 L
BioProcess BPG 300/500 glass column	10 cm	7.1 L

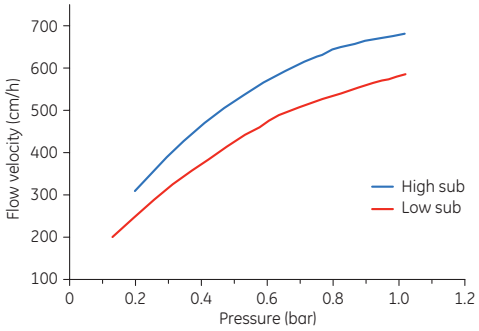


Fig 2. Typical pressure/flow curves for Phenyl Sepharose 6 Fast Flow (low sub) and Phenyl Sepharose 6 Fast Flow (high sub) in an XK 50/30 Column, bed height 15 cm; mobile phase 0.1 M NaCl.

Table 1. Medium characteristics

Characteristics	low sub	high sub
Degree of substitution	approx. 20 µmol phenyl per mL gel	approx. 40 µmol phenyl per mL gel
Mean particle size	90 µm	
Bead size range	45 to 165 µm	
Bead structure	highly cross-linked agarose, 6%, spherical	
Linear flow velocity at 25°C in XK 50/30 Column, 15 cm bed height in 0.1 M NaCl	≥ 400 cm/h at 1 bar (100 kPa, 14.5 psi)	
Recommended pH working range	3 to13	
pH stability, cleaning-in-place	2 to14	
Chemically stable for 7 d at 40°C in	1 M NaOH, 3 M ammonium sulphate, 70% ethanol, 30% isopropanol, 10% ethylene glycol, 0.5% SDS, 6 M guanidine hydrochloride, and 8 M urea	
Autoclavable	121°C for 20 minutes in H_20	

Application

Human alpha-fetoprotein (αFP) plays an important role in fetal development and in cancer. The chromatograms in Figure 3 show a successful two-step purification procedure for a monoclonal antibody (IgG_1) raised against αFP. The hybridoma was cultured in a hollow fibre reactor and the monoclonal antibody isolated, in high yield, on Phenyl Sepharose 6 Fast Flow (high sub). In the second step, contaminating albumin was removed by gel filtration using Superdex™ 200 prep grade medium.

Process hygiene

Good process hygiene enables safety and integrity of the final product by removing or controlling any unwanted substances that might be present or generated in the raw material, or derived from the purification system itself. Good process hygiene also has a positive effect on process economy by preventing successive build-up of contaminating material on the separation medium, thus prolonging the life of the packed column.

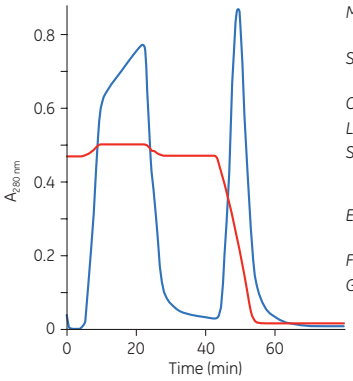
Regeneration

Wash with two bed volumes of water, followed by two to three bed volumes of starting buffer. A complete cleaning-in-place (CIP) procedure is recommended after approximately five runs, depending on the starting material.

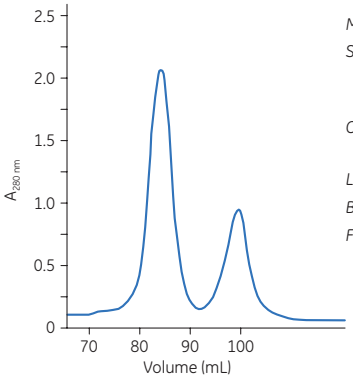
CIP

A CIP procedure is the removal from the purification system of very tightly bound precipitated or denaturared substances generated in previous production runs. In some applications, substances such as lipids or denatured proteins may remain in the column bed, and not be eluted by the regeneration procedure. A specific CIP protocol has to be designed according to the type of contaminants known to be present in the feed stream. Recommended procedures for the removal of these contaminants without dismantling the column are described below. Column performance is not significantly changed by CIP procedures for at least 100 CIP cycles.

1. **Protocol to 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.
2. **Protocol 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. (Apply gradients to avoid air bubble formation when using high concentration of organic solvents.)
   - Alternatively, wash the column with detergent in a basic or acidic solution. For example, use 0.5% non-ionic detergent in 1 M acetic acid. Wash at a flow velocity of 40 cm/h. Residual detergent can be removed by washing with 5 bed volumes of 70% ethanol.



Medium: Phenyl Sepharose 6 Fast Flow (high sub)
Sample: Harvest from hollow fibre containing 4% fetal calf serum
Column: XK 16/30, 20 mL medium volume
Loading: 4 mg MAb/mL medium
Starting buffer: 0.6 M $(NH_4)_2SO_4$ + 20 mM K_2HPO_4, pH 7.0
Elution buffer: 20 mM K_2HPO_4, pH 7.0
Flow rate: 10 mL/min (300 cm/h)
Gradient volume: 10 column volumes



Medium: Superdex 200 prep grade
Sample: Gradient eluted material from Phenyl Sepharose 6 Fast Flow (high sub)
Column: XK 50/100, 1700 mL medium volume
Loading: 7 mL (11 mg protein/mL)
Buffer: 0.9% NaCl
Flow rate: 10 mL/min (30 cm/h)

Electrophoresis: PhastGel™ 10% to 15% polyacrylamide, SDS, nonreducing conditions

Lane 1 Low molecular weight markers
Lane 2 Harvest from hollow fibre
Lane 3 Flow through material from Phenyl Sepharose 6 Fast Flow (high sub)
Lane 4 Eluted material from Phenyl Sepharose 6 Fast Flow (high sub)
Lane 5 MAb from Superdex 200 prep grade polishing
Lane 6 Peak #2 from Superdex 200 prep grade polishing
Lane 7
Lane 8 Low molecular weight markers

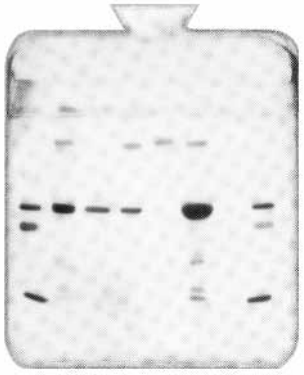


Fig 3. Purification procedure for a monoclonal antibody (MAb).

Figure 4 shows the results of a clearance study, a test which determines the amount of water required to replace the storage buffer containing 20% ethanol.

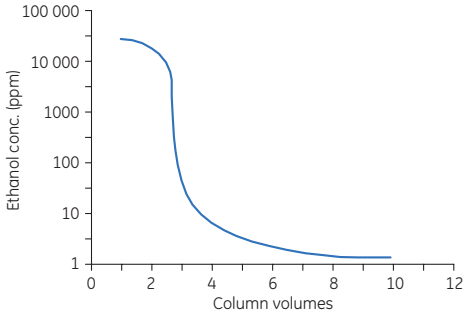


Fig 4. Removal of 20% ethanol from Phenyl Sepharose 6 Fast Flow (high sub) in an HR 10/10 Column, bed volume 8 mL; mobile phase H_2O; flow rate 1 mL/min.

Sanitization and sterilization

Sanitization using NaOH reduces microbial contamination of the medium bed to a minimum, without dismantling the column. The CIP procedures recommended above also sanitize Phenyl Sepharose Fast Flow media effectively. A concentration of 0.5 to 1.0 M NaOH with a contact time of 30 to 60 min has proved effective for most microbial contaminations.

For sterilization of the chromatography media, dismantle the column and autoclave the media at 121°C for 20 min. Remember to sterilize the column parts before re-assembling and packing the column.

Operation

Phenyl Sepharose 6 Fast Flow (low sub) and Phenyl Sepharose 6 Fast Flow (high sub) are supplied preswollen in 20% ethanol.

Equilibrate the HIC media in three volumes of working buffer before packing.

We recommend that the sample be passed through a 0.22 to 0.45 µm filter to prolong the life of the separation media.

Elution conditions have to be optimized for different samples to obtain maximum purity and throughput.

Storage

For longer periods of storage (e.g., weeks), we recommend that the media be stored at 3°C to 8°C in 20% ethanol.

References

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Ordering information

Product	Pack size	Code number
Phenyl Sepharose 6 Fast Flow (low sub)	200 mL	17-0965-05
Phenyl Sepharose 6 Fast Flow (low sub)	1 L	17-0965-03
Phenyl Sepharose 6 Fast Flow (low sub)	5 L	17-0965-04
Phenyl Sepharose 6Fast Flow (high sub)	200 mL	17-0973-05
Phenyl Sepharose 6Fast Flow (high sub)	1 L	17-0973-03
Phenyl Sepharose 6Fast Flow (high sub)	5 L	17-0973-04

For local office contact information, visit
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