



## Hydrophobic interaction chromatography

# HiPrep™ Phenyl FF (high sub) 16/10 HiPrep Phenyl FF (low sub) 16/10 HiPrep Butyl FF 16/10 HiPrep Octyl FF 16/10

Four prepacked HiPrep 20 mL columns are available for hydrophobic interaction chromatography (HIC). The columns are designed for convenient preparative separations of proteins and other biomolecules on the basis of their varying hydrophobic interactions with hydrophobic groups attached to the uncharged resin. GE's HIC resins have distinct hydrophobic characteristics that cover most application and development work.

### Key features include:

- Simple operation and high binding capacity
- Reliable and reproducible separations
- Excellent for scale-up from HiTrap™ or HiScreen™ prepacked columns
- Compatible with single pump-based configurations or chromatography systems such as ÄKTA™ systems

## Hydrophobic interaction chromatography

HIC is widely used as an initial or intermediate step in separation and purification of biomolecules such as proteins and polypeptides. The technique is based on the hydrophobic interactions between the solute biomolecules and immobilized hydrophobic groups. As biological molecules differ in hydrophobicity, separation is based on the varying strength of the hydrophobic interactions. Binding is promoted by the presence of moderately high concentrations



**Fig 1.** HiPrep Phenyl FF (high sub) 16/10, HiPrep Phenyl FF (low sub) 16/10, HiPrep Butyl FF 16/10, and HiPrep Octyl FF 16/10 columns.

of anti-chaotropic salts and elution is improved by decreasing the affinity of the biomolecules for the hydrophobic groups on the resin, usually by decreasing the ionic strength of the elution buffer.

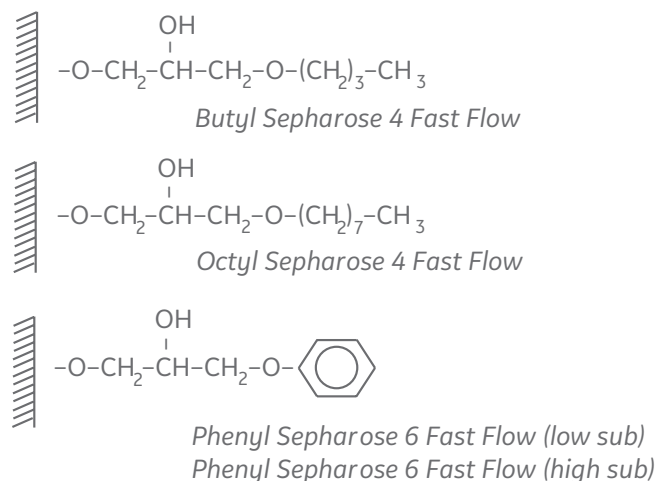
Some of the separation and purification parameters that must be considered for optimizing binding, resolution, and selectivity are: sample characteristics, ligand structure and density, type of base matrix, salt concentration and type of salt, flow rate, temperature, and pH.

As the hydrophobic behavior of biomolecules is difficult to predict, the parameters listed above must be selected empirically. The different hydrophobic characteristics of HiPrep HIC columns offer the user the possibility to find the resin that corresponds to their specific needs (Fig 1).

## Chromatography resin characteristics

HiPrep Phenyl FF (high sub) 16/10, HiPrep Phenyl FF (low sub) 16/10, HiPrep Butyl FF 16/10, and HiPrep Octyl FF 16/10 are packed with Sepharose™ Fast Flow resins. The resins are based on a rigid, cross-linked ~90 µm beaded agarose matrix with good flow properties. The functional groups are coupled to the matrix via chemically stable glycidyl ether linkages resulting in uncharged matrices (Fig 2).

All resins have excellent chemical and physical properties, allowing harsh working and cleaning-in-place conditions. Resin characteristics are summarized in Table 1.



**Fig 2.** Functional groups of Sepharose Fast Flow HIC resins.

## Column characteristics

HiPrep columns are made of polypropylene, which is biocompatible with biomolecules. The columns are easily connected to a variety of chromatographic systems, including simple pump-based configurations and ÄKTA systems. Column characteristics are summarized in Table 2. The columns are not designed to be opened or repacked.

**Table 2.** Characteristics of HiPrep 16/10 columns

Column dimension	1.6 × 10.0 cm
Bed volume	20 mL
Recommended operating flow rate*	2–10 mL/min
Maximum operating flow rate*	10 mL/min
Maximum operating pressure	1.5 bar (0.15 MPa, 22 psi)
Column hardware pressure limit	5 bar (0.5 MPa, 73 psi)

\* At room temperature using water.

**Table 1.** Resin characteristics of Phenyl (high sub), Phenyl (low sub), Butyl, and Octyl Sepharose Fast Flow

	Phenyl (high sub)	Phenyl (low sub)	Butyl	Octyl
Matrix	Cross-linked agarose, 6%, spherical	Cross-linked agarose, 6%, spherical	Cross-linked agarose, 4%, spherical	Cross-linked agarose, 4%, spherical
Particle size, $d_{50V}$ *	~90 µm	~90 µm	~90 µm	~90 µm
Hydrophobic ligand	Phenyl	Phenyl	Butyl	Octyl
Ligand concentration (µmol/mL resin)	~ 45	~ 25	~ 40	~ 5
pH stability				
pH stability, CIP <sup>†</sup>	2–14	2–14	2–14	2–14
pH stability, operational <sup>†</sup>	3–13	3–13	3–13	3–13
Chemical stability	Stable to commonly used aqueous buffers, 1.0 M NaOH <sup>‡</sup> , 6 M guanidine hydrochloride, 70% ethanol, and 30% isopropanol			
Storage	4°C to 30°C in 20% ethanol or 0.01 M NaOH <sup>§</sup>	4°C to 30°C in 20% ethanol or 0.01 M NaOH <sup>§</sup>	4°C to 30°C in 20% ethanol or 0.01 M NaOH <sup>§</sup>	4°C to 30°C in 20% ethanol or 0.01 M NaOH <sup>§</sup>

\* Median particle size of the cumulative volume distribution.

<sup>†</sup> pH range where resin can be operated without significant change in function.

<sup>‡</sup> 1.0 M NaOH should only be used for cleaning purposes.

<sup>§</sup> In most cases, no long term stability data has been generated by GE Healthcare Life Sciences in 0.01 M NaOH. In some cases, accelerated studies at elevated temperature indicate that storage in 0.01 M NaOH can be a viable option but no guarantees can be made regarding retained function of the product.

Columns: HiPrep Phenyl FF (high sub) 16/10,  
HiPrep Phenyl FF (low sub) 16/10,  
HiPrep Butyl FF 16/10, and HiPrep Octyl FF 16/10\*

Samples: 1. Cytochrome C (10 mg/mL)  
2. Ribonuclease A (30 mg/mL)  
3. Lysozyme (10 mg/mL)  
4.  $\alpha$ -chymotrypsinogen (10 mg/mL)

Sample volume: 2 mL

Binding buffer: 100 mM sodium phosphate, 1.5 M  $(\text{NH}_4)_2\text{SO}_4$  pH 7.0

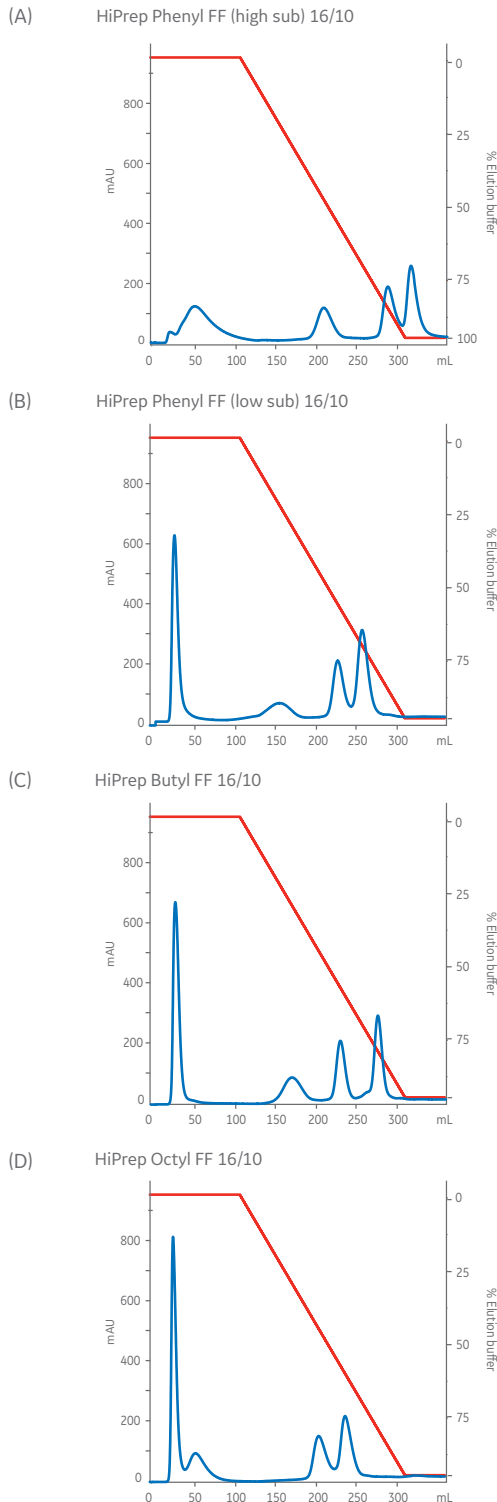
Elution buffer: 100 mM sodium phosphate, pH 7.0

Flow rate: 2 mL/min, 60 cm/h

Gradient: 0% to 100% elution buffer in 200 mL (10 CV)

System: ÄKTAexplorer

Detection: 280 nm



**Fig 3 A to D.** Comparison of the selectivity of the HiPrep 16/10 HIC columns using four model proteins.

## Applications

HiPrep Phenyl FF (high sub) 16/10, HiPrep Phenyl FF (low sub) 16/10, HiPrep Butyl FF 16/10, and HiPrep Octyl FF 16/10 can be used for a wide range of initial and intermediate purification applications, alone or together with other chromatographic techniques.

Figure 3 shows the separation of four model proteins under identical experimental conditions. The different elution profiles reflect the broad range of selectivity of the columns, and can serve as a guideline for choosing the most suitable resin for a wide range of applications.

Figure 4 shows partial purification of alkaline phosphatase from *E. coli* homogenate using HiPrep Phenyl FF (high sub) 16/10 for the initial capture step. By selecting conditions where the target molecule binds to the column while most of the contaminants do not bind, the enzyme could be efficiently purified.

Column: HiPrep Phenyl FF (high sub) 16/10\*

Sample: 30 mL centrifuged, filtered, and desalted *E. coli* homogenate

Binding buffer: 100 mM sodium phosphate, 1.5 M  $(\text{NH}_4)_2\text{SO}_4$ , pH 7.0

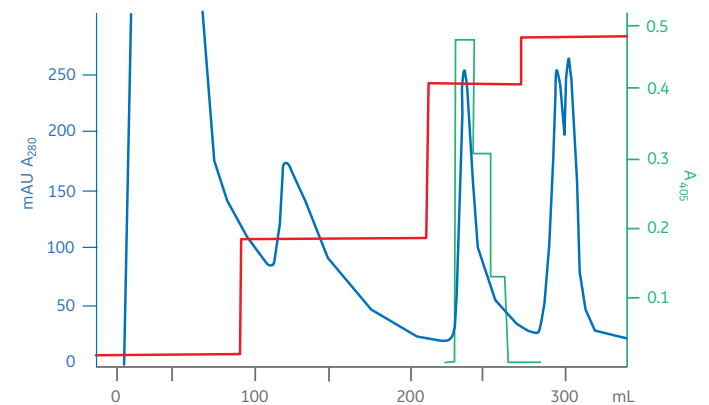
Elution buffer: 100 mM sodium phosphate, pH 7.0

Flow rate: 5 mL/min, 150 cm/h

Gradient: 0% to 37% elution buffer in 0 CV (column volumes)  
37% to 80% elution buffer in 5 CV  
80% to 100% elution buffer in 3 CV

System: ÄKTAexplorer

\* Note: Data was obtained using first-generation HiPrep 16/10 column



**Fig 4.** Partial purification of alkaline phosphatase from *E. coli* on HiPrep Phenyl FF (high sub) 16/10. The green line represents the alkaline phosphatase activity.

## Storage

HiPrep HIC columns are supplied in 20% ethanol. The columns should be stored in 20% ethanol at 4°C to 30°C.

## Ordering information

Product	Quantity	Code number
HiPrep Phenyl FF (high sub) 16/10	1 × 20 mL	28936545
HiPrep Phenyl FF (low sub) 16/10	1 × 20 mL	28936546
HiPrep Butyl FF 16/10	1 × 20 mL	28936547
HiPrep Octyl FF 16/10	1 × 20 mL	28936548

### Related products

HiTrap HIC Selection Kit, 7 different HIC resins	7 × 1 mL	28411007
HiTrap Phenyl FF (high sub)	5 × 1 mL	17135501
	5 × 5 mL	17519301
HiTrap Phenyl FF (low sub)	5 × 1 mL	17135301
	5 × 5 mL	17519401
HiTrap Phenyl HP	5 × 1 mL	17135101
	5 × 5 mL	17519501
HiTrap Butyl HP	5 × 1 mL	28411001
	5 × 5 mL	28411005
HiTrap Butyl FF	5 × 1 mL	17135701
	5 × 5 mL	17519701
HiTrap Butyl-S FF	5 × 1 mL	17097813
	5 × 5 mL	17097814
HiTrap Octyl FF	5 × 1 mL	17135901
	5 × 5 mL	17519601
HiLoad™ 16/10 Phenyl Sepharose HP	1 × 20 mL	17108501
HiLoad 26/10 Phenyl Sepharose HP	1 × 53 mL	17108601
HiTrap Desalting	5 × 5 mL	17140801
HiPrep 26/10 Desalting	1 × 53 mL	17508701
	4 × 53 mL	17508702
Phenyl Sepharose High Performance	75 mL*	17108201
Phenyl Sepharose 6 Fast Flow (low sub)	25 mL	17096510
	200 mL*	17096505
Phenyl Sepharose 6 Fast Flow (high sub)	25 mL	17097310
	200 mL*	17097305
Butyl Sepharose 4 Fast Flow	25 mL	17098010
	200 mL*	17098001
Octyl Sepharose 4 Fast Flow	25 mL	17094610
	200 mL*	17094602

\* Larger quantities are available. Please contact your local GE representative.

Accessories	Quantity	Code number
Union M6 female/1/16" male	5	18385801
HiTrap/HiPrep 1/16" male connector for ÄKTA systems	8	28401081

### Related literature

Hydrophobic Interaction and Reversed Phase Chromatography Handbook, Principles and methods	11001269
Prepacked chromatography columns for ÄKTA systems, Selection guide	28931778

## gelifesciences.com/protein-purification

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Butyl-S Sepharose 6 Fast Flow: Separating Miraculin with this product is subject to US patent number

5,886,155. Licenses are available from BioResources International, Inc., Somerset, NJ, USA.

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