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 media. GE Healthcare's HIC media 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™ design

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

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.

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 medium that corresponds to their specific needs (Fig 1).

Chromatography media 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 media. The media are based on a rigid, highly 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 media have excellent chemical and physical properties, allowing harsh working and cleaning-in-place conditions. Media characteristics are summarized in Table 1.

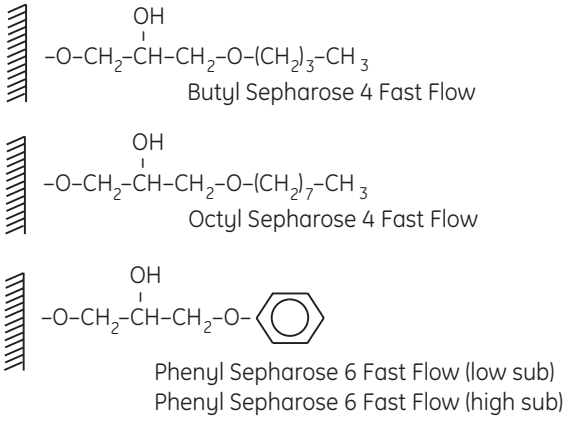


Fig 2. Functional groups of Sepharose Fast Flow HIC media.

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 design. 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 flow rate[1]	2–10 ml/min (60–300 cm/h)
Maximum flow rate[1]	10 ml/min (300 cm/h)
Maximum pressure over the packed bed during operation	1.5 bar (0.15 MPa, 22 psi)
Column hardware pressure limit	5 bar (0.5 MPa, 73 psi)

[1] Water at room temperature.

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

	Phenyl (high sub)	Phenyl (low sub)	Butyl	Octyl
Matrix	6% highly cross-linked, spherical agarose	6% highly cross-linked, spherical agarose	4% highly cross-linked, spherical agarose	4% highly cross-linked, spherical agarose
Mean particle size	90 µm	90 µm	90 µm	90 µm
Hydrophobic ligand	Phenyl	Phenyl	Butyl	Octyl
Ligand density (µmol/ml medium)	40	20	50	5
pH stability				
short term[1]	2–14	2–14	2–14	2–14
long term and working range[2]	3–13	3–13	3–13	3–13
Chemical stability	All commonly used buffers (pH 3–13), 1 M NaOH, 8 M urea, 6 M guanidine hydrochloride, 70% ethanol, and 30% isopropanol			
Storage	4°C to 30°C in 20% ethanol or 0.01 M NaOH	4°C to 30°C in 20% ethanol or 0.01 M NaOH	4°C to 30°C in 20% ethanol or 0.01 M NaOH	4°C to 30°C in 20% ethanol or 0.01 M NaOH

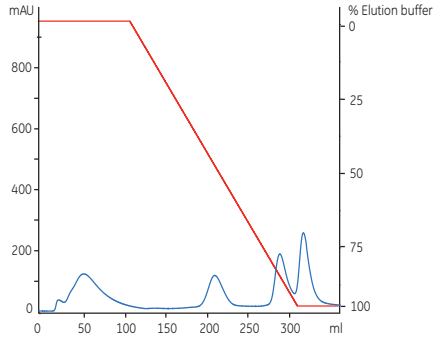
[1] Refers to regeneration

[2] Refers to conditions where the medium is stable over a long period of time

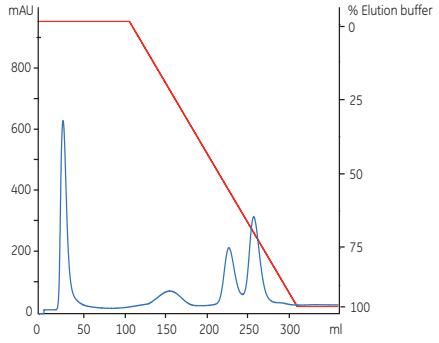
Columns:	HiPrep Phenyl FF (high sub) 16/10, HiPrep Phenyl FF (low sub) 16/10, HiPrep Butyl FF 16/10, and HiPrep Octyl FF 16/10*
Samples:	1. Cytochrome C (10 mg/ml) 2. Ribonuclease A (30 mg/ml) 3. Lysozyme (10 mg/ml) 4. α-chymotrypsinogen (10 mg/ml)
Sample volume:	2 ml
Binding buffer:	100 mM sodium phosphate, 1.5 M $(NH_4)_2SO_4$ 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

* Note: Data was obtained using first-generation HiPrep 16/10 columns.

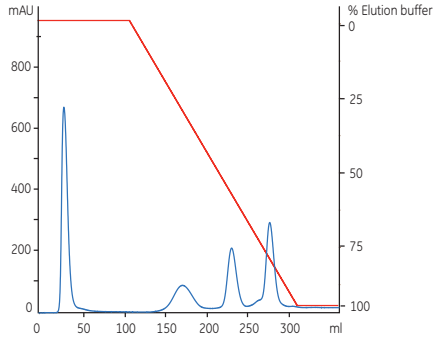
A) **HiPrep Phenyl FF (high sub) 16/10**



B) **HiPrep Phenyl FF (low sub) 16/10**



C) **HiPrep Butyl FF 16/10**



D) **HiPrep Octyl FF 16/10**

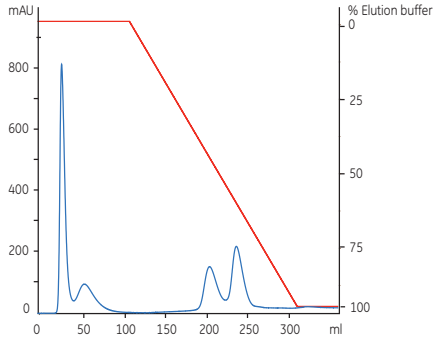


Fig 3 A-D. Comparison of the selectivity of the HiPrep 16/10 HIC columns using four model proteins.

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 medium 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 $(NH_4)_2SO_4$, 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% elution buffer in 5 CV 37% to 80% elution buffer in 0 CV 80% elution buffer in 3 CV 80% to 100% elution buffer in 0 CV
System:	ÄKTAexplorer

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

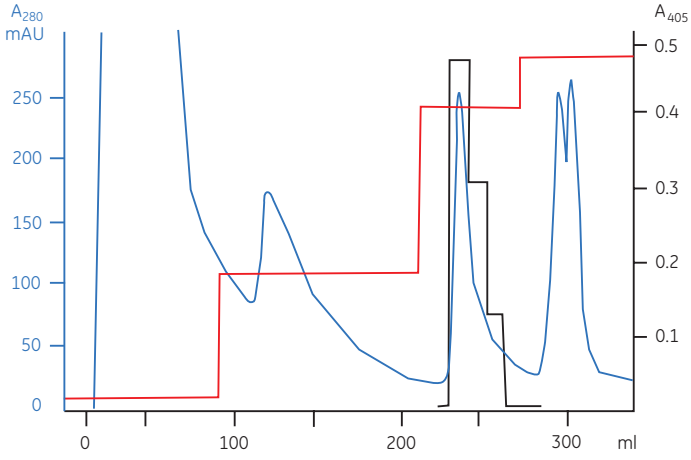


Fig 4. Partial purification of alkaline phosphatase from *E. coli* on HiPrep Phenyl FF (high sub) 16/10. The black line represents the alkaline phosphatase activity.

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 No.
HiPrep Phenyl FF (high sub) 16/10	1 × 20 ml	28-9365-45
HiPrep Phenyl FF (low sub) 16/10	1 × 20 ml	28-9365-46
HiPrep Butyl FF 16/10	1 × 20 ml	28-9365-47
HiPrep Octyl FF 16/10	1 × 20 ml	28-9365-48

Related products	Quantity	Code No.
HiTrap HIC Selection Kit, 7 different HIC media	7 × 1 ml	28-4110-07
HiTrap Phenyl FF (high sub)	5 × 1 ml	17-1355-01
	5 × 5 ml	17-5193-01
HiTrap Phenyl FF (low sub)	5 × 1 ml	17-1353-01
	5 × 5 ml	17-5194-01
HiTrap Phenyl HP	5 × 1 ml	17-1351-01
	5 × 5 ml	17-5195-01
HiTrap Butyl HP	5 × 1 ml	28-4110-01
	5 × 5 ml	28-4110-05
HiTrap Butyl FF	5 × 1 ml	17-1357-01
	5 × 5 ml	17-5197-01
HiTrap Butyl-S FF	5 × 1 ml	17-0978-13
	5 × 5 ml	17-0978-14
HiTrap Octyl FF	5 × 1 ml	17-1359-01
	5 × 5 ml	17-5196-01
HiLoad™ 16/10 Phenyl Sepharose HP	1 × 20 ml	17-1085-01
HiLoad 26/10 Phenyl Sepharose HP	1 × 53 ml	17-1086-01
HiTrap Desalting	5 × 5 ml	17-1408-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
	4 × 53 ml	17-5087-02

Related products	Quantity	Code No.
Phenyl Sepharose High Performance	75 ml[1]	17-1082-01
Phenyl Sepharose 6 Fast Flow (low sub)	25 ml	17-0965-10
	200 ml[1]	17-0965-05
Phenyl Sepharose 6 Fast Flow (high sub)	25 ml	17-0973-10
	200 ml[1]	17-0973-05
Butyl Sepharose 4 Fast Flow	25 ml	17-0980-10
	200 ml[1]	17-0980-01
Octyl Sepharose 4 Fast Flow	25 ml	17-0946-10
	200 ml[1]	17-0946-02

[1] Larger quantities are available. Please contact your local GE Healthcare representative.

Accessories	Quantity	Code No.
Union M6 female/1/16" male	5	18-3858-01
HiTrap/HiPrep 1/16" male connector for ÄKTA design	8	28-4010-81

Related literature	Code No.
Hydrophobic Interaction and Reversed Phase Chromatography Handbook, Principles and Methods	11-0012-69
Prepacked chromatography columns for ÄKTA design systems, Selection Guide	28-9317-78

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