GE Healthcare

Instructions 28-4026-54 AB

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

HiPrep™ 16/10 Phenyl FF (high sub), HiPrep 16/10 Phenyl FF (low sub), HiPrep 16/10 Butyl FF, and HiPrep 16/10 Octyl FF are prepacked, ready to use columns for hydrophobic interaction chromatography (HIC). The columns provide fast, preparative separations of proteins and other biomolecules based on their hydrophobic interaction with hydrophobic groups attached to the uncharged gel. See table below for column characteristics.

Column data

Matrix	6% highly cross-linked spherical agarose (Phenyl) 4% highly cross-linked spherical agrose (Butyl, Octyl)
Mean particle size	90 µm
Bed volume	20 ml
Bed height	100 mm
i.d.	16 mm
Column composition	Polypropylen
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, Δp[2]	0.15 MPa, 1.5 bar, 22 psi
HiPrep column hardware pressure limit[2]	0.5 MPa, 5 bar, 73 psi
Storage	+4 °C to +30 °C in 20% ethanol

	Phenyl (high sub)	Phenyl (low sub)	Butyl	Octyl
Hydrophobic ligand	Phenyl	Phenyl	Butyl	Octyl
Ligand density (µmol/ml medium)	40	20	50	5
pH stability				
short term	2–14	2–14	2–14	2–14
long term and working range	3–13	3–13	3–13	3–13

[1] Water at room temperature. Flow rate is determined by $v \bullet \eta \leq 10$ ml/min where v = flow rate and η = viscosity.

[2] Many chromatography systems are equipped with pressure gauges to measure the pressure at a particular point in the system, usually just after the pumps. The pressure measured here is the sum of the pre-column pressure, the pressure drop over the medium bed, and the post-column pressure. It is always higher than the pressure drop over the bed alone. We recommend keeping the pressure drop over the bed below 1.5 bar. Setting the upper limit of your pressure gauge to 1.5 bar will ensure the pump shuts down before the medium is overpressured.

If necessary, post-column pressure of up to 3.5 bar can be added to the limit without exceeding the column hardware limit. To determine post-column pressure, proceed as follows:

To avoid breaking the column, the post-column pressure must never exceed 3.5 bar.

1. Connect a piece of tubing in place of the column.
2. Run the pump at the maximum flow you intend to use for chromatography. Use a buffer with the same viscosity as you intend to use for chromatography. Note the back pressure as total pressure.
3. Disconnect the tubing and run at the same flow rate used in step 2. Note this back pressure as pre-column pressure.
4. Calculate the post-column pressure as total pressure minus pre-column pressure.

If the post column pressure is higher than 3.5 bar, take steps to reduce it (shorten tubing, clear clogged tubing, or change flow restrictors) and perform steps 1–4 again until the post column pressure is below 3.5 bar. When the post column pressure is satisfactory, add the post column pressure to 1.5 bar and set this as the upper pressure limit on the chromatography system.

First time use

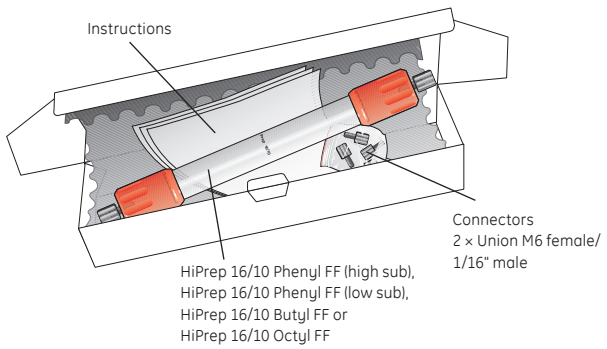
Ensure an appropriate pressure limit has been set.

Equilibrate the column for first time use or after long-term storage by running:

a) 100 ml elution buffer (low salt) at 5 ml/min (see section "Choice of elution buffer" recommendations).

b) 100 ml of start buffer (high salt) at 5 ml/min.

These HiPrep columns can be used directly on ÄKTAdesign™ system without the need for extra connectors.



Try these conditions first

Hydrophobic interaction chromatography is usually performed with moderately high concentrations of salts in the start buffer (salt promotes adsorption) and elution is achieved by a linear or stepwise decrease in concentration of the salt.

Start buffer:	0.05 M sodium phosphate buffer, 1 M $(NH_4)_2SO_4$, pH 7.0
Elution buffer:	0.05 M sodium phosphate buffer, pH 7.0
Flow rate:	5 ml/min at room temperature
Gradient:	0–100% elution buffer in 200 ml (10 CV)

Equilibration before a new run

Proceed according to the instructions in section "First time use". Please read the back of these instructions for information on optimizing a separation.

Buffers and solvent resistance

De-gas and filter all solutions through a 0.45 µm filter toincrease column lifetime.

Daily use
All commonly used aqueous buffers, pH 3–13
Guanidine hydrochloride, up to 6 M
Urea, up to 8 M (not tested for butyl and octyl media)

Cleaning

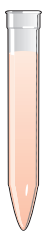
Sodium hydroxide, up to 1 M
Ethanol, up to 70%
Isopropanol up to 30%

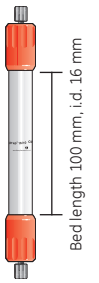
Avoid

Solutions <pH 2
Phenol

Sample preparation

Dissolve the sample in start buffer (high salt), filter through 0.45 µm or centrifuge at 10 000 × g for 10 min.





Delivery/storage

The column is supplied in 20% ethanol. If the column is to be stored for more than two days after use, clean the column according to the procedure described under "Cleaning-in-Place (CIP)". Then equilibrate with at least 100 ml of 20% ethanol or 0.01 M NaOH at a flow rate of 5 ml/min.

DO NOT OPEN THE COLUMN!

Choice of buffer

All standard aqueous buffers can be used.

When selecting salt for the start buffer refer to the Hofmeister series (see below). Increasing the salting-out effect strengthens the hydrophobic interactions, whereas increasing the chaotropic effect weakens them.

← **Increasing 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 chaotrophic effect →

HiPrep

Table 1 lists volatile buffers used in cases where the purified substance has to be freeze-dried.

Table 1. Volatile buffer systems

pH	Substances
2.3–3.5	Pyridine/formic acid
3.0–5.0	Trimethylamine/formic acid
4.0–6.0	Trimethylamine/acetic acid
6.8–8.8	Trimethylamine/HCl
7.0–8.5	Ammonia/formic acid
8.5–10.0	Ammonia/acetic acid
7.0–12.0	Trimethylamine/CO_2
8.0–9.5	Ammonium carbonate/ammonia
8.5–10.5	Ethanolamine/HCl

Optimization

Perform your first run according to "Try these conditions first".

If the obtained results are unsatisfactory, consider the following:

Action	Effect
Change salt concentration	Higher salt concentration increases retention time
	Lower salt concentration decreases retention times
Increase gradient volume	May improve resolution
Decrease flow rate	Improves resolution
Increase temperature	Increases retention times
Change pH	Changes selectivity
Increase salt concentration	May increase capacity
Change to a medium with a different ligand	Selectivity change

Cleaning-in-place (CIP)

Regular cleaning

Regenerate the column after each run by rinsing it with 100 ml distilled water at a flow rate of 5 ml/min at room temperature to elute material still bound to the column.

Re-equilibrate the column with at least 100 ml start buffer at a flow rate of 5 ml/min at room temperature until the UV baseline and pH/conductivity values are stable.

More rigorous cleaning

Reverse the flow direction and run the following sequence of solutions at a flow rate of 5 ml/min at room temperature:

1. 80 ml of a 1 M NaOH solution (removes precipitated proteins, hydrophobically bound proteins, and lipoproteins from the column) followed by 80 ml distilled water.
2. 80 ml of 70% ethanol or 30% isopropanol (removes proteins, lipoproteins, and lipids that are strongly hydrophobically bound to the column) followed by 60 ml distilled water.

After cleaning, equilibrate the column with approximately 100 ml start buffer at a flow rate of 5 ml/min at room temperature before use.

DO NOT OPEN THE COLUMN!

Trouble shooting

Symptom	Remedy
Increased backpressure	Reverse the flow direction and pump 100 ml elution buffer at a over the column flow rate of 5 ml/min at room temperature through the column. Return to normal flow direction and run 100 ml buffer at a flow rate of 5 ml/min through the column. If backpressure is not decreased reverse the flow direction again and follow the rigorous cleaning instructions.
Loss of resolution and/or decreased sample recovery	Follow the procedure described in the section "More rigorous cleaning".
Air in the column	Reverse the flow direction and pump 100 ml of well de-gassed start buffer through the column at a flow rate of 5 ml/min at room temperature.

Column performance control

We recommend checking column performance at regular intervals. The function of the column can be checked as described in Figure 1.

Sample:	Cytochrome C (10 mg/ml) Ribonuclease A (30 mg/ml) Lysozyme (10 mg/ml) α-chymotrypsinogen (10 mg/ml)
Sample volume:	2 ml
Start 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/hr (room temperature)
Gradient:	0–100% elution buffer in 200 ml (10 CV)
Instrumentation:	ÄKTAexplorer™

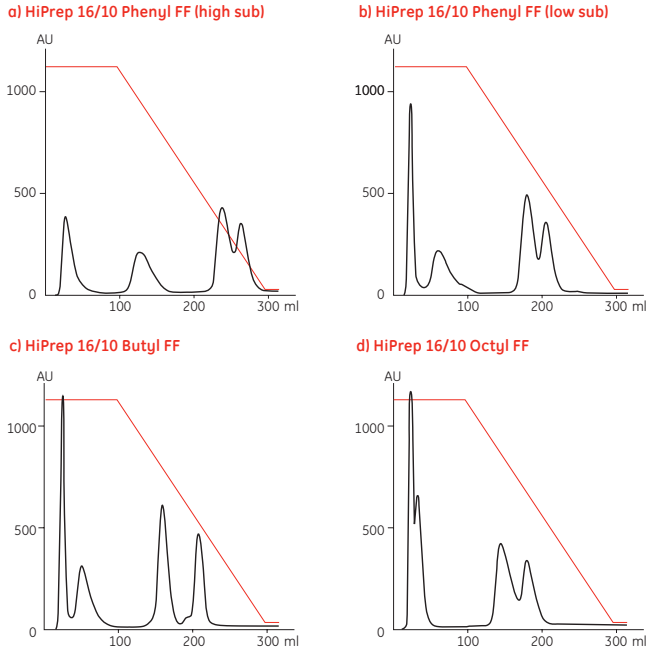


Figure 1. Typical chromatogram from a function test of **(a)** HiPrep 16/10 Phenyl FF (high sub), **(b)** HiPrep 16/10 Phenyl FF (low sub), **(c)** HiPrep 16/10 Butyl FF and **(d)** HiPrep 16/10 Octyl FF.

Ordering information

Designation	No. per pack	Code No.
HiPrep 16/10 Phenyl FF (high sub)	1 (20 ml)	17-5095-01
HiPrep 16/10 Phenyl FF (low sub)	1 (20 ml)	17-5094-01
HiPrep 16/10 Butyl FF	1 (20 ml)	17-5096-01
HiPrep 16/10 Octyl FF	1 (20 ml)	17-5097-01

Companion products

HiPrep 26/10 Desalting	1 (53 ml)	17-5087-01
HiPrep 26/10 Desalting	4 (53 ml)	17-5087-02
HiTrap™ HIC Selection Kit	6 × 1 ml	11-0034-53
HiTrap Phenyl FF (high sub)	5 × 1 ml	17-1355-01
HiTrap Phenyl FF (high sub)	5 × 5 ml	17-5193-01
HiTrap Phenyl FF (low sub)	5 × 1 ml	17-1353-01
HiTrap Phenyl FF (low sub)	5 × 5 ml	17-5194-01
HiTrap Phenyl HP	5 × 1 ml	17-1351-01
HiTrap Phenyl HP	5 × 5 ml	17-5195-01
HiTrap Octyl FF	5 × 1 ml	17-1359-01
HiTrap Octyl FF	5 × 5 ml	17-5196-01
HiTrap Butyl FF	5 × 1 ml	17-1357-01
HiTrap Butyl FF	5 × 5 ml	17-5197-01
HiTrap Butyl-S FF	5 × 1 ml	17-0978-13
HiTrap Butyl-S FF	5 × 5 ml	17-0978-14

Accessories

HiTrap/HiPrep 1/16" male connector for ÄKTAdesign	8	24-4010-81
To connect columns with 1/16" connections to FPLC™ system: Union M6 female/1/16" male*	5	18-3858-01
Handbook, Hydrophobic Interaction Chromatography and Reversed Phase Chromatography, Principles & Methods		11-0012-69

* 2 units (red polypropylene) are included in HiPrep package

Further information

Check www.gehealthcare.com/protein-purification for more information.

www.gehealthcare.com/protein-purification
www.gehealthcare.com

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28-4026-54 AB 08/2006

Elanders Österväla 2006