HiTrap HIC Selection Kit

Introduction

The HiTrap™ HIC Selection Kit consists of seven hydrophobic interaction chromatography (HIC) media with different hydrophobic characteristics. The kit provides you with the possibility to screen for the most appropriate HIC media to use for specific application and development work. The seven different HIC media are prepacked in ready to use 1 ml HiTrap columns. Separations are easily performed with a syringe, a pump or a chromatography system such as ÄKTAdesign™.

- Convenient and fast to use
- Simple operation
- Hydrophobic interaction media screening
- Easy to scale-up

Fig 1. HiTrap HIC Selection Kit now including seven different HiTrap HIC 1-ml columns.

Hydrophobic interaction chromatography

Substances are separated on the basis of their varying strengths of hydrophobic interactions with hydrophobic groups attached to an uncharged matrix. This technique is usually performed in the presence of moderately high concentrations of salts in the adsorption buffer (these salts promote adsorption and may have a stabilizing influence on protein structure). Elution is achieved by a linear or stepwise decrease in concentration of the salt. Several factors influence the chromatographic behavior of proteins and peptides on hydrophobic adsorbents. Parameters that influence binding, resolution, selectivity, and recovery include:

- Ligand structure and ligand density
- Type of base matrix
- Sample characteristics
- Flow rate
- Type and concentration of salt
- Temperature

The practical implication of these effects is that different HIC media must be compared individually. The choice of ligand, type, and concentration of salt and pH are all empirical and must be established by screening experiments for each separation.

Media characteristics

The HIC media packed in the HiTrap columns are based on the highly cross-linked beaded agarose matrices, Sepharose™ Fast Flow, and Sepharose High Performance, which have excellent flow properties and high physical and chemical stability. The HIC ligands are coupled to the monosaccharide units via their corresponding glycidyl ethers, giving matrices without charges and stable ether bonds between the ligands and the agarose. The ligands are shown in Table 1.

Table 1. Characteristics of HiTrap HIC media

Media[1]	Hydrophobic ligand		Ligand density (µmol/ml medium)	Average particle size (µm)	pH stability Short term[2]	pH stability Long term[3]
Phenyl Sepharose High Performance	Phenyl	—O— 	25	34	2–14	3–13
Phenyl Sepharose 6 Fast Flow (low sub)	Phenyl	—O— 	25	90	2–14	3–13
Phenyl Sepharose 6 Fast Flow (high sub)	Phenyl	—O— 	40	90	2–14	3–13
Butyl Sepharose High Performance	Butyl	—O—$(CH_2)_3$—CH_3	50	34	2–14	3–13
Butyl Sepharose 4 Fast Flow[4]	Butyl	—O—$(CH_2)_3$—CH_3	40	90	2–14	3–13
Butyl-S Sepharose 6 Fast Flow	Butyl-S	—S—$(CH_2)_3$—CH_3	10	90	2–14	3–13
Octyl Sepharose 4 Fast Flow[4]	Octyl	—O—$(CH_2)_7$—CH_3	5	90	2–14	3–13

[1] Storage: 0.01 M NaOH or 20% ethanol.

[2] pH stability, short term: pH interval to which the medium can be subjected for cleaning- or sanitization-in-place (accumulated 90-100 hours at room temperature) without significant change in function.

[3] pH stability, long term: pH interval where the medium can be operated without significant change in function.

[4] Matrices: All media are based on spherical, 6% cross-linked agarose beads except for Butyl Sepharose 4 Fast Flow and Octyl Sepharose 4 Fast Flow, which are based on spherical, 4% cross-linked agarose beads.

Table 2. Chemical stability of HIC media.

	Phenyl Sepharose High Performance	Phenyl Sepharose 6 Fast Flow (low sub)	Phenyl Sepharose 6 Fast Flow (high sub)	Butyl Sepharose High Performance	Butyl Sepharose 4 Fast Flow	Butyl-S Sepharose 6 Fast Flow	Octyl Sepharose 4 Fast Flow
1 M NaOH	X	X	X	X	X	X	X
1 M acetic acid	X	n.d.	n.d.	X	n.d.	n.d.	n.d.
1 mM HCl	n.d.	n.d.	n.d.	X	X	X	X
3 M $(NH_4)SO_4$	n.d.	X	X	X	n.d.	X	n.d.
70% ethanol	X	X	X	X	X	X	X
30% isopropanol	X	X	X	X	X	X	X
6 M guanidine hydrochloride	X	X	X	X	X	X	X
8 M urea	X	X	X	X	n.d.	X	n.d.

[X] =functionally stable for 7 days at 40°C. n.d. = not determinded

HiTrap HIC Selection Kit consists of the following seven prepacked HIC media from GE Healthcare. Each has different hydrophobic characteristics.

- Phenyl Sepharose High Performance
- Phenyl Sepharose 6 Fast Flow (low sub)
- Phenyl Sepharose 6 Fast Flow (high sub)
- Butyl Sepharose High Performance
- Butyl Sepharose 4 Fast Flow
- Butyl-S Sepharose 6 Fast Flow
- Octyl Sepharose 4 Fast Flow

Characteristics of HiTrap HIC media are listed in Table 1 and their chemical stability is shown in Table 2.

Column characteristics

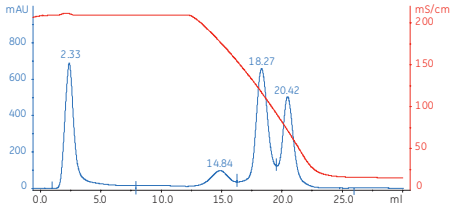
The characteristics of HiTrap columns are shown in Table 3. The columns are made of bio-inert polypropylene. The column is delivered with a stopper on the inlet and a snap-off end on the outlet. Connectors for using the columns with a syringe, laboratory pump, or a chromatography system are included in the package. Note that HiTrap columns cannot be opened or refilled.

Table 3. Characteristics of HiTrap 1-ml column

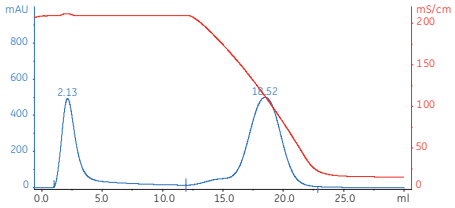
Recommended flow rate	1.0 ml/min
Maximum flow rate	4.0 ml/min
Column dimensions	0.7 × 2.5 cm
Column volume	1 ml
Maximum backpressure	3 bar, 42 psi, 0.3 MPa

Sample: Cytochrome C, Ribonuclease A, Lysozyme, α-chymotrypsinogen 6 mg protein/ml, (1:3:1:1) in start buffer
Column volume: 1 ml
Sample volume: 1 ml
Sample load: 6 mg protein/ml medium
Flow rate: 1.0 ml/min, (150 cm/h)
Start buffer (A): 0.1 M Na_2HPO_4, 1.7 M $(NH_4)_2SO_4$, pH 7.0
Elution buffer (B): 0.1 M Na_2HPO_4, pH 7.0
Gradient: 0%–100% Elution buffer in 10 ml
System: ÄKTA$_{FPLC}$™

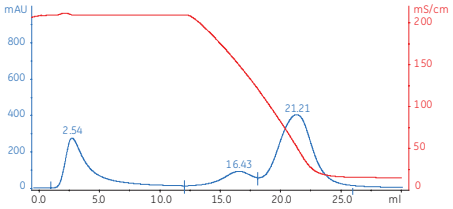
Phenyl Sepharose High Performance



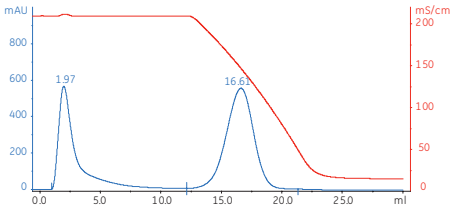
Phenyl Sepharose 6 Fast Flow (low sub)



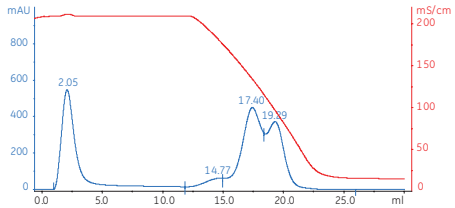
Phenyl Sepharose 6 Fast Flow (high sub)



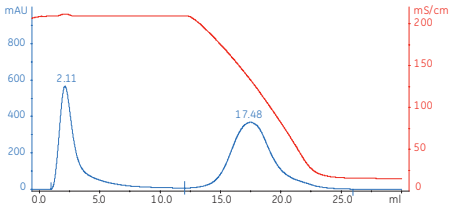
Butyl-S Sepharose 6 Fast Flow



Butyl Sepharose 4 Fast Flow



Octyl Sepharose 4 Fast Flow



Butyl Sepharose High Performance

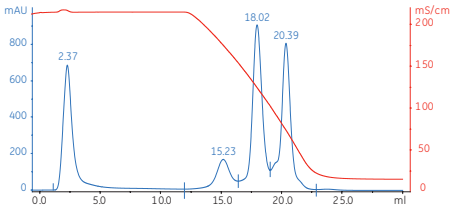


Fig 2. Comparison of the selectivity of the different media in HiTrap HIC Selection Kit. Elution volumes at each peak.

Operation

Complete, easy-to-follow instructions are included for fast startup, media, and method optimization. Separations can be easily achieved using a syringe for stepwise elution, or a pump or a liquid chromatography system such as ÄKTAdesign for gradient applications. A set of connectors is supplied for connection of the column to different types of equipment.

For quick scale-up of purifications, two or three HiTrap HIC columns of the same type can be connected in series. Further scale-up can be achieved using the prepacked columns HiPrep™ 16/10 Phenyl FF (high sub), HiPrep 16/10 Phenyl FF (low sub), HiPrep 16/10 Butyl FF, or HiPrep 16/10 Octyl FF. Prepacked HiLoad™ Phenyl Sepharose HP or bulk media packs are also available, see ordering information.

Regeneration of HIC adsorbents is normally done by washing with distilled water. To prevent slow build up of contaminants on the column over time, regular cleaning is advised. Precipitated proteins can be removed by washing with 0.5–1.0 M NaOH followed by distilled water. Strongly bound substances can be removed by washing with up to 70% ethanol or 30% isopropanol.

For longer periods of storage, the columns should be filled with 20% ethanol or 0.01 M NaOH and stored at 4°C to 30°C.

Applications

Screening

The effects of the different hydrophobic characteristics of the seven HIC media are shown in Figure 2. Model proteins were separated using the same method and buffers. After sample injection and washing, the bound proteins were eluted with a decreasing gradient over 10 ml.

Another example is shown in Figure 3, where Ribonuclease A and β-lactoglobulin were separated on the seven HIC media using the same method. The different media were ranked according to increasing elution volume for Ribonuclease A. As can be seen, the ranking is completely different for β-lactoglobulin, indicating differences in selectivity, the largest differences are demonstrated for Octyl Sepharose 4 Fast Flow and Butyl-S Sepharose 6 Fast Flow.

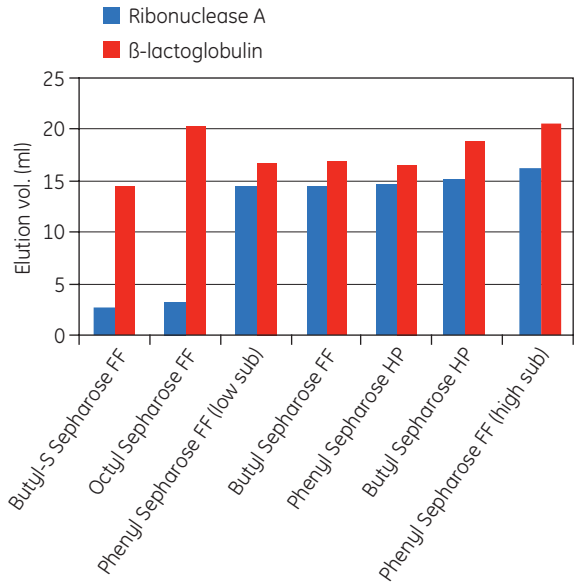


Fig 3. Comparison of elution volume for Ribonuclease A and β-lactoglobulin. The different HIC media are arranged after increasing elution volumes for Ribonuclease A.

Sample:	Cytochrome C, Ribonuclease A, Lysozyme, α-chymotrypsinogen 6 mg protein/ml, (1:3:1:1) in start buffer
Column volume:	1 ml
Sample volume:	1 ml
Flow rate:	0.5 ml/min, (75 cm/h)
Start buffer (A):	0.1 M Na_2HPO_4,
Containing in A	1.7 M $(NH_4)_2SO_4$, pH 7.0
Containing in B	1.0 M Na_2SO_4, pH 7.0
Containing in C	3.0 M NaCl, pH 7.0
Elution buffer (B):	0.1 M Na_2HPO_4, pH 7.0
Gradient:	0%–100% Elution buffer in 10 ml
Detection:	UV-M, 5 mm cell, 280 nm, 1.0 AUFS

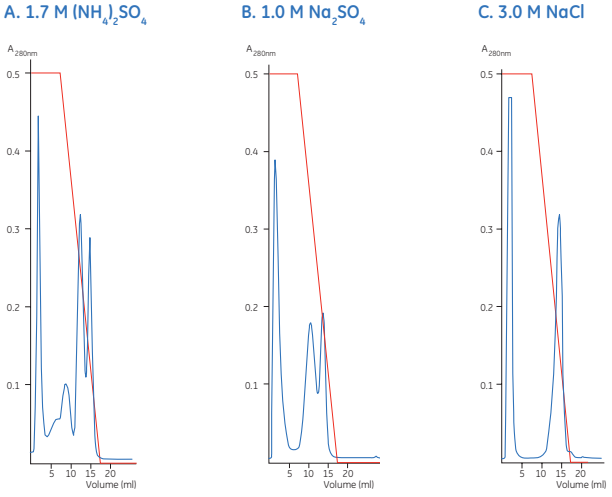


Fig 4 A–C. Effect of different salts on selectivity and resolution when the same sample was separated on Butyl Sepharose 4 Fast Flow.

Effect of different salts

The most frequently used salts in HIC are ammonium sulfate and sodium sulfate. "Weaker" salts such as sodium chloride may also be considered. The effect of these salts on the separation is shown in Figure 4. The same sample was separated on Butyl Sepharose 4 Fast Flow with the different salts, ammonium sulfate, sodium sulfate and sodium chloride. The type of salt and its concentration have a profound effect on the chromatographic separation.

Ordering information

Product	Quantity	Code No.
HiTrap HIC Selection Kit, seven different HIC media	7 × 1 ml	28-4110-07

Related products	Quantity	Code No.
Prepacked columns		
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 Butyl HP	5 × 1 ml	28-4110-01
HiTrap Butyl HP	5 × 5 ml	28-4110-05
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
HiTrap Octyl FF	5 × 1 ml	17-1359-01
HiTrap Octyl FF	5 × 5 ml	17-5196-01
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
HiLoad 16/10 Phenyl Sepharose HP	1 (20 ml)	17-1085-01
HiLoad 26/10 Phenyl Sepharose HP	1 (53 ml)	17-1086-01

Bulk media	Quantity	Code No.
Phenyl Sepharose High Performance	75 ml[1]	17-1082-01
Phenyl Sepharose 6 Fast Flow (low sub)	25 ml 200 ml[1]	17-0965-10 17-0965-05
Phenyl Sepharose 6 Fast Flow (high sub)	25 ml 200 ml[1]	17-0973-10 17-0973-05
Butyl Sepharose High Performance	25 ml 200 ml[1]	17-5432-01 17-5432-02
Butyl Sepharose 4 Fast Flow	25 ml 200 ml[1]	17-0980-10 17-0980-01
Butyl-S Sepharose 6 Fast Flow	25 ml 200 ml[1]	17-0978-10 17-0978-02
Octyl Sepharose 4 Fast Flow	25 ml 200 ml[1]	17-0946-10 17-0946-02

[1] Larger quantities are available. Please contact GE Healthcare for more information.

Accessories	Quantity	Code No.
1/16" male/luer female[1]	2	18-1112-51
Tubing connector flangeless/ M6 female[1]	2	18-1003-68
Tubing connector flangeless/ M6 male[1]	2	18-1017-98
Union 1/16" female/M6 male[1]	6	18-1112-57
Union M6 female /1/16" male[1]	5	18-3858-01
Union luerlock female/M6 female	2	18-1027-12
HiTrap/HiPrep, 1/16" male connector for ÄKTAdesign	8	28-4010-81
Stop plug female, 1/16"[2]	5	11-0004-64
Fingertight stop plug, 1/16"[3]	5	11-0003-55

[1] One connector included in each HiTrap package.

[2] Two, five, or seven stop plugs female included in HiTrap packages depending on the product.

[3] One fingertight stop plug is connected to the top of each HiTrap column at delivery.

Literature	Code No.
Hydrophobic Interaction Chromatography & Reversed Phase Chromatography, Principles and Methods, Handbook	11-0012-69
Convenient Protein Purification, HiTrap Column Guide	18-1129-81

Please check *www.gehealthcare.com/hitrap* or contact your local representative for further information.

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