HiTrap HIC Selection Kit

HYDROPHOBIC INTERACTION CHROMATOGRAPHY

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

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

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

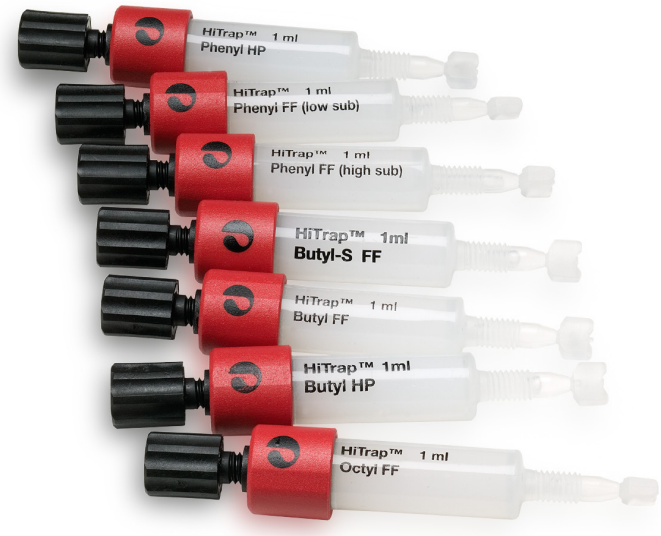

Fig 1. HiTrap HIC Selection Kit includes seven different HiTrap HIC 1 mL columns.

The practical implication of these effects is that different HIC resins 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.

Resin characteristics

The HIC resins 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 monosacharide 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.

HiTrap HIC Selection Kit consists of the following seven prepacked HIC resins from Cytiva. 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 resins are listed in Table 1 and their chemical stability is shown in Table 2.

Table 1. Characteristics of HiTrap HIC resins

Resin[1]	Hydrophobic ligand		Ligand concentration (µmol/mL resin)	Particle size, d_{50v} (µm)[2]	pH stability, CIP[3]	pH stability, operational [4]
Phenyl Sepharose High Performance	Phenyl	—O— 	~ 25	~ 34	3 to 12	3 to 12
Phenyl Sepharose 6 Fast Flow (low sub)	Phenyl	—O— 	~ 25	~ 90	2 to 14	3 to 13
Phenyl Sepharose 6 Fast Flow (high sub)	Phenyl	—O— 	~ 45	~ 90	2 to 14	3 to 13
Butyl Sepharose High Performance	Butyl	—O—$(CH_2)_3$—CH_3	~ 50	~ 34	2 to 14	3 to 13
Butyl Sepharose 4 Fast Flow[5]	Butyl	—O—$(CH_2)_3$—CH_3	~ 40	~ 90	2 to 14	3 to 13
Butyl-S Sepharose 6 Fast Flow	Butyl-S	—O—$(CH_2)_3$—CH_3	~ 10	~ 90	2 to 14	3 to 13
Octyl Sepharose 4 Fast Flow[5]	Octyl	—O—$(CH_2)_7$—CH_3	~ 5	~ 90	2 to 14	3 to 13

[1] Storage: 20% ethanol
[2] Median particle size of the cumulative volume distribution
[3] pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function
[4] pH range where resin can be operated without significant change in function
[5] Matrices: All resins 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 resins

	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
1.0 M NaOH[1]	n.d.	×	×	×	×	×	×
1 M acetic acid	×	n.d.	n.d.	×	n.d.	n.d.	n.d.
1 mM HCl	×	n.d.	n.d.	×	×	×	×
3 M $(NH_4)SO_4$[2]	n.d.	×	×	×	n.d.	×	n.d.
70% ethanol	×	×	×	×	×	×	×
30% isopropanol	×	×	×	×	×	×	×
6 M guanidine hydrochloride	×	×	×	×	×	×	×
8 M urea	×	×	×	×	n.d.	×	n.d.
0.01 M NaOH	×	×	×	×	×	×	×

× = functionally stable n.d. = not determined
[1] 1.0 M NaOH should only be used for cleaning purposes
[2] Due to instability, ammonium sulphate is not suitable when working at pH values above 8.0

Column characteristics

The characteristics of HiTrap columns are shown in Table 3. The columns are made of polypropylene, a biocompatible material that does not interact with biomolecules. The column is delivered with a stopper on the inlet and a snapoff end on the outlet. Note that HiTrap columns cannot be opened or refilled.

Table 3. Characteristics of HiTrap 1 mL column

Recommended operating flow rate	1.0 mL/min
Maximum operating flow rate	4.0 mL/min
Column dimensions	0.7 × 2.5 cm
Column volume	1 mL
Column hardware pressure limit	5 bar (0.5MPa, 70 psi)

Operation

Complete, easy-to-follow instructions are included for fast startup, resin, and method optimization. Separations can be easily achieved using a syringe for stepwise elution, or a pump or a liquid chromatography system such as an ÄKTA system for gradient applications.

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 resin 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 sodium hydroxide 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 and stored at 4°C to 30°C.

Applications

Screening

The effects of the different hydrophobic characteristics of the seven HIC resins 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.

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 resin
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% to 100% elution buffer in 10 mL
System: ÄKTA$_{FPLC}$

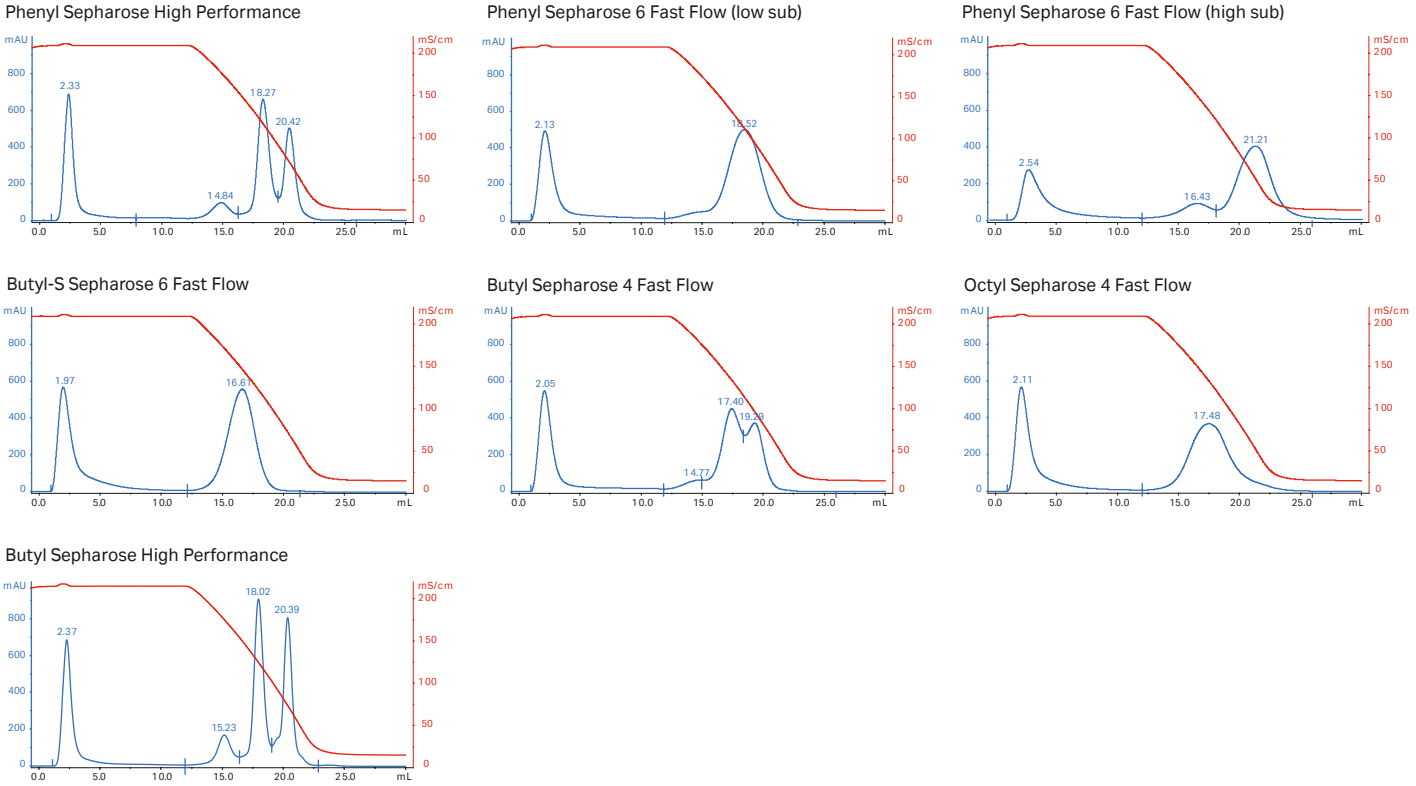

Fig 2. Comparison of the selectivity of the different resins in HiTrap HIC Selection Kit. Elution volumes at each peak.

Another example is shown in Figure 3, where Ribonuclease A and β-lactoglobulin were separated on the seven HIC resins using the same method. The different resins 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.

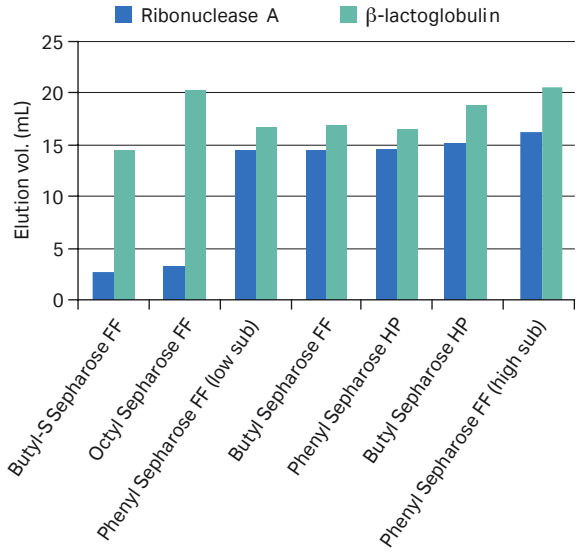

Fig 3. Comparison of elution volume for Ribonuclease A and β-lactoglobulin. The different HIC resins are arranged after increasing elution volumes for Ribonuclease A.

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.

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% to 100% elution buffer in 10 mL
Detection:	UV-M, 5 mm cell, 280 nm, 1.0 AUFS

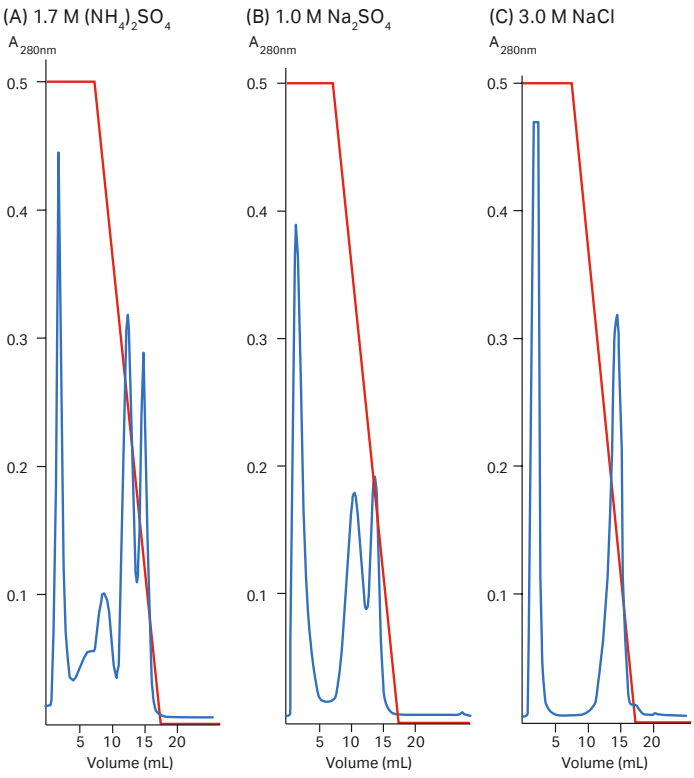

Fig 4. Effect of different salts on selectivity and resolution when the same sample was separated on Butyl Sepharose 4 Fast Flow.

Ordering information

Product	Quantity	Code number
HiTrap HIC Selection Kit, seven different HIC resins	7 × 1 mL	28411007

Prepacked columns	Quantity	Code number
HiTrap Phenyl FF (high sub)	5 × 1 mL	17135501
HiTrap Phenyl FF (high sub)	5 × 5 mL	17519301
HiTrap Phenyl FF (low sub)	5 × 1 mL	17135301
HiTrap Phenyl FF (low sub)	5 × 5 mL	17519401
HiTrap Phenyl HP	5 × 1 mL	17135101
HiTrap Phenyl HP	5 × 5 mL	17519501
HiTrap Butyl HP	5 × 1 mL	28411001
HiTrap Butyl HP	5 × 5 mL	28411005
HiTrap Butyl FF	5 × 1 mL	17135701
HiTrap Butyl FF	5 × 5 mL	17519701
HiTrap Butyl-S FF	5 × 1 mL	17097813
HiTrap Butyl-S FF	5 × 5 mL	17097814
HiTrap Octyl FF	5 × 1 mL	17135901
HiTrap Octyl FF	5 × 5 mL	17519601
HiPrep 16/10 Phenyl FF (high sub)	1 (20 mL)	17509501
HiPrep 16/10 Phenyl FF (low sub)	1 (20 mL)	17509401
HiPrep 16/10 Butyl FF	1 (20 mL)	17509601
HiPrep 16/10 Octyl FF	1 (20 mL)	17509701
HiLoad 16/10 Phenyl Sepharose HP	1 (20 mL)	17108501
HiLoad 26/10 Phenyl Sepharose HP	1 (53 mL)	17108601

Bulk resin	Quantity	Code number
Phenyl Sepharose High Performance	75 mL[1]	17108201
Phenyl Sepharose 6	25 mL	17096510
Fast Flow (low sub)	200 mL[1]	17096505
Phenyl Sepharose 6	25 mL	17097310
Fast Flow (high sub)	200 mL[1]	17097305
Butyl Sepharose	25 mL	17543201
High Performance	200 mL[1]	17543202
Butyl Sepharose 4 Fast Flow	25 mL 200 mL[1]	17098010 17098001
Butyl-S Sepharose 6 Fast Flow	25 mL 200 mL[1]	17097810 17097802
Octyl Sepharose 4 Fast Flow	25 mL 200 mL[1]	17094610 17094602

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

Accessories

	Quantity	Code number
1/16" male/Luer female*	2	18111251
Tubing connector flangeless/M6 female	2	18100368
Tubing connector flangeless/M6 male	2	18101798
Union 1/16" female/M6 male	6	18111257
Union M6 female/1/16" male	5	18385801
Union luerlock female/M6 female	2	18102712
HiTrap/HiPrep, 1/16" male connector for ÄKTA systems	8	28401081
Stop plug female, 1/16"†	5	11000464
Fingertight stop plug, 1/16"‡	5	11000355

* One connector included in each HiTrap package
† Two, five, or seven stop plugs female included in HiTrap packages depending on products
‡ One fingertight stop plug is connected to the top of each HiTrap column at delivery

Related literature

	Code number
Hydrophobic Interaction Chromatography and Reversed Phase Chromatography, Principles and Methods, Handbook	11001269

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