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If you have questions about applying methodology described in this article to a current application, please contact our technical service chemists.



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Fast, Convenient Protein Preparation: HiTrap™ HIC and Ion Exchange LC Cartridges

K. Pardue

Amersham Pharmacia Biotech HiTrap cartridges are designed for low-pressure hydrophobic interaction or ion exchange applications for proteins. The HiTrap HIC Test Kit consists of five media, with different hydrophobic characteristics, packed in ready-to-use 1mL HiTrap cartridges. Reliable and highly reproducible Q Sepharose High Performance (a strong anion exchange medium) and SP Sepharose High Performance (a strong cation exchange medium) gels are packed in 1mL and 5mL HiTrap ion exchange cartridges. Both media have high loading capacities and remain charged over broad pH ranges. HiTrap cartridges are convenient, fast, and, although disposable, very affordable. They enable you to separate biomolecules rapidly—usually in minutes.

Few protein purification tools are as convenient, fast, simple, and inexpensive to use as Pharmacia® HiTrap cartridges (Figure A). Designed for low-pressure hydrophobic interaction and ion exchange applications for proteins, these ready-to-use 1mL and 5mL plastic cartridges with syringe and pump connections are packed with functionalized gels. Although disposable, the cartridges are very affordable. The highly crosslinked base material, Sepharose®, is compatible with high flow rates, enabling you to separate biomolecules rapidly—usually in minutes. A luer adapter and complete instructions are included with each package of cartridges.

Figure A. HiTrap Cartridges



995-0119

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Hydrophobic Interaction Chromatography

Ligand type and degree of substitution, salt type and concentration, pH, and other variables influence protein binding by hydrophobic interaction chromatography (HIC). These parameters must be established by experiment for each new separation.* Because the nature of HIC makes it difficult to predict the optimum medium for a specific application, the HiTrap HIC Test Kit is ideal for screening and method development, especially for process development in industry. This kit consists of five HIC media, with different hydrophobic characteristics, packed in 1mL HiTrap cartridges. The media are: Phenyl Sepharose High Performance, Phenyl Sepharose 6 Fast Flow (low sub), Phenyl Sepharose 6 Fast Flow (high sub), Butyl Sepharose 4 Fast Flow, and Octyl Sepharose 4 Fast Flow. The HIC ligands are coupled to the monosaccharide units via their corresponding glycidyl ethers, producing uncharged matrices with stable ether bonds between the ligands and the agarose. Packing and cartridge characteristics are summarized in Table 1.

Table 1. Characteristics of HiTrap HIC Cartridges

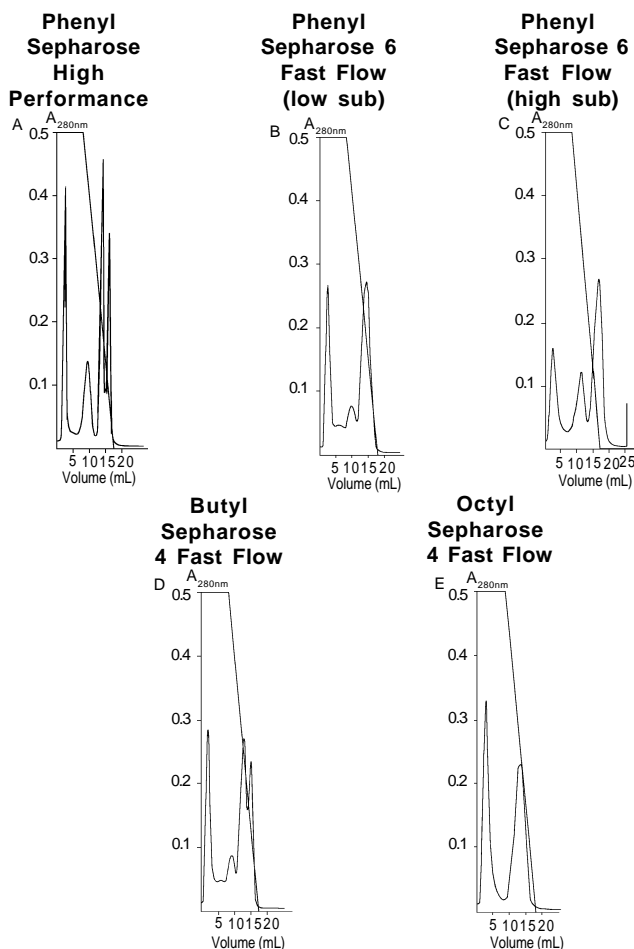
Phenyl Sepharose High Performance	
Ligand:	phenyl, 25µmol/mL gel
Packing:	6% crosslinked spherical agarose, 24-44µm diameter (mean = 34µm)
Phenyl Sepharose 6 Fast Flow (low sub)	
Ligand:	phenyl, 20µmol/mL gel
Packing:	6% crosslinked spherical agarose, 45-165µm diameter (mean = 90µm)
Phenyl Sepharose 6 Fast Flow (high sub)	
Ligand:	phenyl, 40µmol/mL gel
Packing:	6% crosslinked spherical agarose, 45-165µm diameter (mean = 90µm)
Butyl Sepharose 4 Fast Flow	
Ligand:	n-butyl, 50µmol/mL gel
Packing:	4% crosslinked spherical agarose, 45-165µm diameter (mean = 90µm)
Octyl Sepharose 4 Fast Flow	
Ligand:	n-octyl, 5µmol/mL gel
Packing:	4% crosslinked spherical agarose, 45-165µm diameter (mean = 90µm)
All Cartridges	
Volume/Dimensions:	1mL (2.5 x 0.7cm)
Recommended Flow Rate:	<2mL/min (Phenyl Sepharose High Performance) <1mL/min (all other cartridges)
Maximum Flow Rate:	4mL/min (all cartridges)
Maximum Back Pressure:	3 bar (42 psi / 0.3MPa)
pH Stability:	2-14 (short term) or 3-13 (long term)
Storage:	0.01M sodium hydroxide or 20% ethanol

*For information on experimental parameters order *Hydrophobic Interaction Chromatography: Principles and Methods* (Catalog No. 23582).

Figure B shows the performances of the five ligands with the same sample, buffers, and 10mL decreasing salt gradient. A clear benefit is the ability to test all five cartridges under the same conditions in less than 2 hours (20-25 min run times). For this application, Phenyl Sepharose HP is clearly the medium of choice. Figure C depicts the elution volumes for ribonuclease A and β -lactoglobulin, in the same mobile phase. The five HIC media are ranked according to increasing elution volume; differences in selectivity are reflected in the ranking. For instance, Octyl Sepharose 4 Fast Flow is least retentive for ribonuclease A, but most retentive for β -lactoglobulin. The information in Figures B and C demonstrates the need for experimentation with each new protein separation.

Figure B. Selectivity of the Media in the HiTrap HIC Test Kit

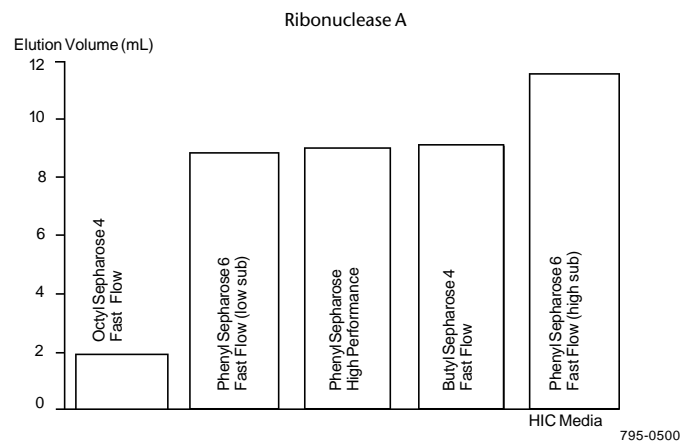
Columns: **HiTrap HIC, 1mL**
 Cat. No.: **54825** (HiTrap HIC Kit)
 Mobile Phase: A = 0.1M Na₂HPO₄/1.7M (NH₄)₂SO₄ (pH 7.0)
 B = 0.1M Na₂HPO₄ (pH 7.0)
 0% B to 100% B over 10mL
 Flow Rate: 1mL/min
 Det.: UV, 280nm
 Inj.: 1mL mobile phase A containing cytochrome c, ribonuclease A, lysozyme, α -chymotrypsinogen, 1:3:1:1 (6mg/mL total)



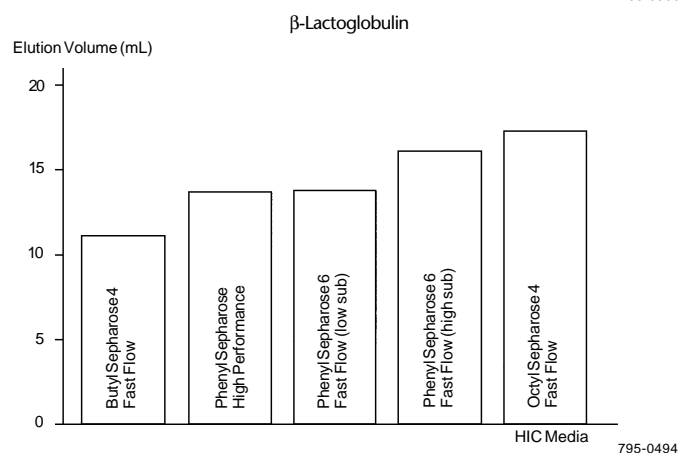
795-0495, 0496, 0497, 0498, 0499

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Figure C. Elution Volumes for Media in the HiTrap HIC Test Kit



795-0500



795-0494

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Ion Exchange Chromatography

The ion exchange media packed in 1mL and 5mL HiTrap ion exchange cartridges are the reliable and highly reproducible Q Sepharose High Performance (a strong anion exchange medium) and SP Sepharose High Performance (a strong cation exchange medium) gels. Both have high loading capacities and remain charged over broad pH ranges. The cartridges are designed for method development, group separations, sample clean-up, and pre-analysis concentration of ionic biomolecules. In many purification schemes, sample concentration is required prior to a gel filtration step, since sample volume is an important factor affecting resolution in size exclusion chromatography. HiTrap ion exchange cartridges are ideal for a first-step clean-up of crude samples, before subsequent purification on larger, more expensive columns. At 1mL/min flow rates, the cartridges maintain a high loading capacity, typically 50mg protein/mL gel. Table 2 summarizes additional packing and cartridge characteristics.

Figure D shows a group separation of human milk proteins on a HiTrap SP cartridge, through a three-step gradient analysis. This figure was produced on an FPLC® system, but the same separation can be achieved by introducing the sample onto the HiTrap cartridge with a syringe. After the sample is applied, the cartridge is washed with 100% buffer A, then 90:10 A:B, then 60:40 A:B, then 100% B. The effluent from each buffer step is collected separately for analysis (e.g., SDS electrophoresis, spectroscopy, etc.).

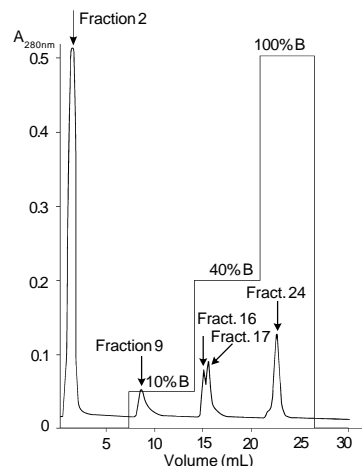
HiTrap HIC and ion exchange cartridges quickly solve many routine purification tasks, with superior results. If you think your application is better suited to fast, simple, affordable affinity chromatography separations, HiTrap affinity cartridges also are available from Supelco.

Table 2. Characteristics of HiTrap Q and HiTrap SP Ion Exchange Cartridges

Packing:	6% highly crosslinked spherical agarose, 34µm
Volume/Dimensions:	1mL (2.5 x 0.7cm) 5mL (2.5 x 1.6cm)
Charged Group:	Q - N ⁺ (CH ₃) ₃ SP - SO ₃ ⁻
Ionic Capacity:	0.14-0.20mmol/mL gel
Dynamic Capacity:	Q - ~50mg human serum albumin/ mL gel (20mM Tris HCl, pH 8.2, 1mL/min) SP - ~55mg ribonuclease/mL gel (0.1M sodium acetate, pH 6.0, 1mL/min)
Recommended Flow Rate:	1mL/min (1mL cartridges) 5mL/min (5mL cartridges)
Maximum Flow Rate:	4mL/min (1mL cartridges) 20mL/min (5mL cartridges)
Maximum Back Pressure:	3 bar (42 psi / 0.3MPa)
Chemical Stability:	all common buffers
pH Stability:	Q - 2-14 (short term) or 2-12 (long term) SP - 3-14 (short term) or 4-13 (long term)
Storage:	Q - 20% ethanol SP - 0.2M sodium acetate in 20% ethanol
Avoid:	oxidizing agents, anionic (Q) or cationic (SP) buffers and detergents

Figure D. Human Milk Proteins by Cation Exchange

Column: **HiTrap SP, 1mL**
 Cat. No.: **54828**
 Mobile Phase: A = 50mM Na acetate (pH 6.0)
 B = 50mM Na acetate/1.0M NaCl (pH 6.0)
 Flow Rate: 1mL/min
 Det.: UV, 280nm
 Inj.: 0.5mL filtered casein-precipitated human milk (buffer exchanged to mobile phase A)



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Ordering Information:

Description	Cat. No.
HiTrap HIC Test Kit	
5 x 1mL cartridges	54814
HiTrap Q Cartridges	
5 x 1mL	54815
5 x 5mL	54816
HiTrap SP Cartridges	
5 x 1mL	54817
5 x 5mL	54818

Suggested Reading:

Description	Cat. No.
Ion Exchange Chromatography: Principles and Methods	23581
Hydrophobic Interaction Chromatography: Principles and Methods	23582
Bulletin 882 <i>Mobile Phases for Ion Exchange and Chromatofocusing</i>	free on request

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