Sephacryl High Resolution media HiPrep Sephacryl HR columns

Sephacryl™ High Resolution (HR) chromatography media allow fast and reproducible purification of proteins, polysaccharides, and other macromolecules by gel filtration at laboratory and industrial scale. Five Sephacryl HR chromatography media are available as prepacked columns and in laboratory and larger pack sizes; Sephacryl S-100 HR, S-200 HR, S-300 HR, S-400 HR, and S-500 HR.

Characteristics of the media include:

- Excellent purification over a wide molecular weight range
- High reproducibility due to high stability
- High flow rates and recoveries
- Well-suited to industrial-scale use

All five Sephacryl HR media are available in prepacked HiPrep™ Sephacryl HR gel filtration columns. Each chromatography medium is available in two different prepacked column sizes; 16/60 (120 ml) and 26/60 (320 ml). HiPrep Sephacryl HR gel filtration columns provide the excellent purification properties of Sephacryl HR media in a convenient, ready-to-use format. The many applications of the columns include preparative purifications.

Characteristics of the columns include:

- Convenient, easy-to-use, prepacked gel filtration columns in two different column sizes
- Choice of five selectivities covering a wide molecular weight range
- Reliable and reproducible preparative purification
- Easy connection to ÄKTAdesign™ chromatography systems

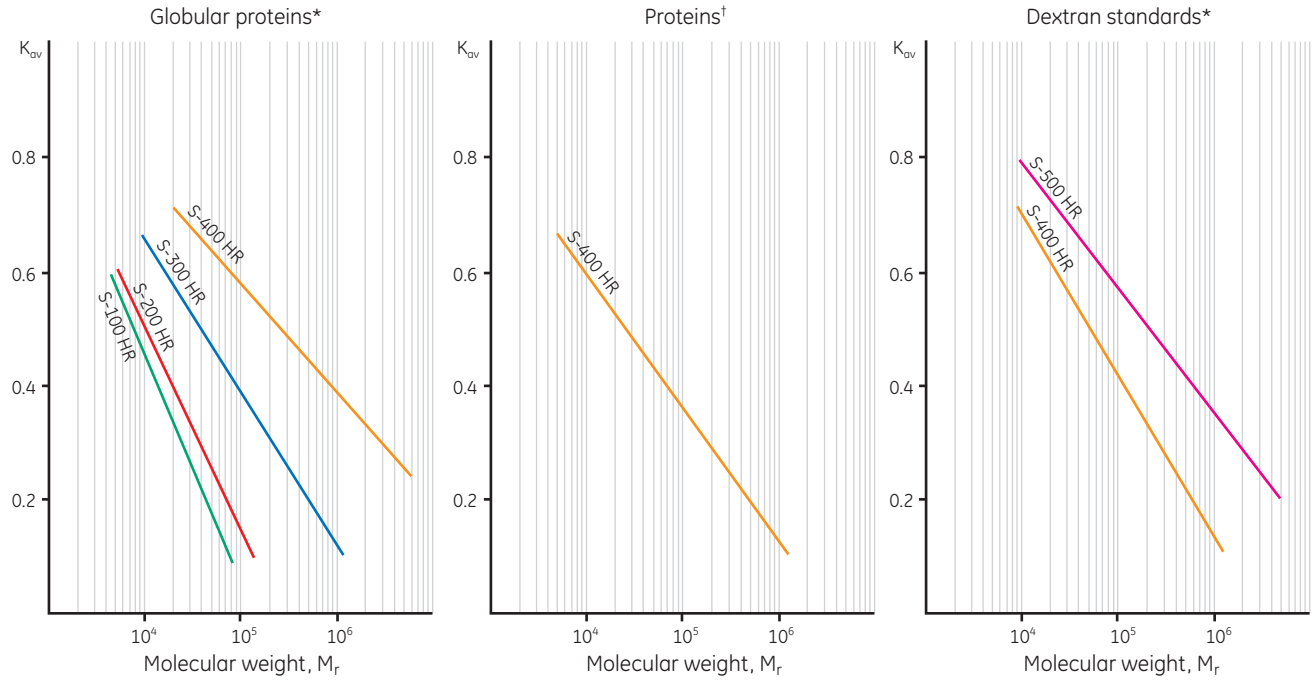
Fig 1. Sephacryl HR chromatography media and HiPrep Sephacryl HR prepacked columns offer the user a wide range of choice and reliable purification by gel filtration.

Media characteristics

The matrix of Sephacryl HR media is a cross-linked copolymer of allyl dextran and N,N'-methylene bisacrylamide. This cross-linking gives good rigidity and chemical stability. The narrow particle size distribution, together with steep selectivity curves, results in good preparative characteristics with maintained high resolution. The hydrophilic nature of the media minimizes nonspecific adsorption and maximizes recovery.

The excellent resolution and flow characteristics, long-term physical and chemical stability, and ease of handling make Sephacryl HR the medium of choice for routine purification.

Sephacryl HR gel filtration media fulfill process chromatography requirements in terms of stability, scalability, and bulk availability. As members of the BioProcess™ family of chromatography media, they carry full technical and regulatory support for production-scale operations.



* In 0.05 M phosphate buffer, 0.15 M NaCl, pH 7.0
† In 6 M guanidine hydrochloride

Fig 2. Selectivity curves for Sephacryl HR chromatography media.

The five chromatography media available have different porosity ranges. Figure 2 shows selectivity curves for each.

For peptides and small proteins, Sephacryl S-100 HR is the best choice. When fractionating proteins in the molecular weight ranges of 5×10^3 to 2.5×10^5 and 1×10^4 to 1.5×10^6, use Sephacryl S-200 HR and Sephacryl S-300 HR, respectively. Note that these ranges include monoclonal antibodies and serum proteins. Sephacryl S-400 HR and Sephacryl S-500 HR are recommended for separating polysaccharides and other macromolecules with extended structures, and even small particles such as plasmids.

Table 1 provides more details on Sephacryl HR media characteristics.

Sephacryl HR media can be packed in most column types, including wide diameter production columns with bed heights of 60 to 100 cm. Equilibration can be performed successfully at high flow rates, which results in fast overall purification runs.

Table 1. Characteristics of Sephacryl HR media

Sephacryl	S-100 HR	S-200 HR	S-300 HR	S-400 HR	S-500 HR
Useful purification range (M_r)					
Globular proteins	1×10^3–1×10^5	5×10^3–2.5×10^5	1×10^4–1.5×10^6	2×10^4–8×10^6	–
Dextrans	–	1×10^3–8×10^4	2×10^3–4×10^5	1×10^4–2×10^6	4×10^4–2×10^7
DNA exclusion limit (base pairs)	–	30	118	271	1078
Average particle size	47 µm	47 µm	47 µm	47 µm	47 µm
Bead structure	Spherical, allyl dextran and N,N'-methylene bisacrylamide				
Chemical stability	Stable in all commonly used buffers: 0.2 M NaOH, 0.1 M HCl, 1 M acetic acid, 8 M urea, 6 M guanidine hydrochloride, 1% SDS, 2 M NaCl, 20% ethanol, 30% propanol, 30% acetonitrile (tested at 40°C for 7 days), 0.5 M NaOH (only for cleaning-in-place)				
pH stability					
long term	3–11	3–11	3–11	3–11	3–11
short term	2–13	2–13	2–13	2–13	2–13
Physical stability	Negligible volume variation due to changes in pH or ionic strength				
Autoclavability	At 121°C, pH 7 for 30 min (media only)				
Storage	20% ethanol	20% ethanol	20% ethanol	20% ethanol	20% ethanol

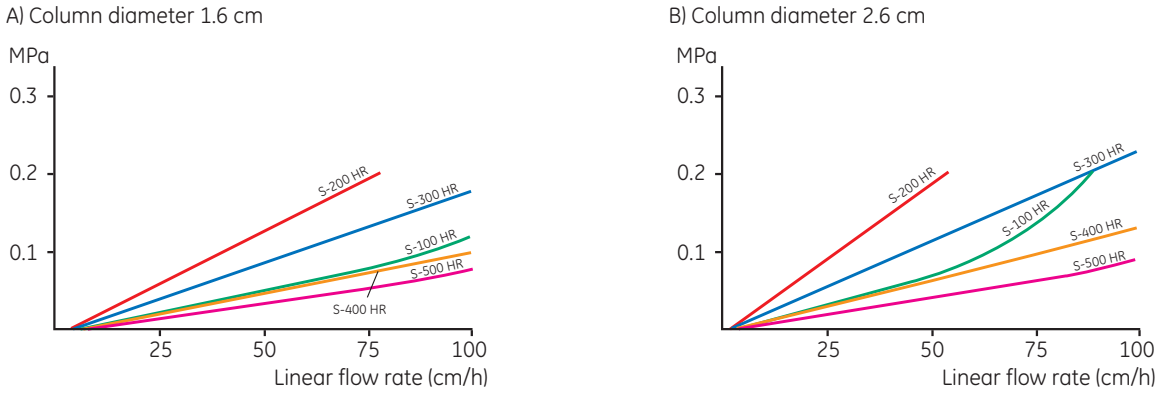


Fig 3. Pressure drop as a function of flow rate for Sephacryl HR media. Bed height approximately 60 cm in distilled water at 25°C. To calculate volumetric flow rate multiply linear flow rate, by the cross-sectional area of column (2 cm^2 for i.d. 16 cm, 5.3 cm^2 for i.d. 26 cm).

Stability

Sephacryl HR is extremely stable, both chemically and physically. The chromatography medium is compatible with all aqueous buffers (from pH 2 to 11) commonly used in biochemistry, and withstands strong bases (e.g., 0.2 M NaOH) and strong acids (e.g., 0.1 M HCl and 1 M acetic acid).

Furthermore, the presence of detergents (e.g., 1% SDS), chaotropic salts or dissociating agents (e.g., 8 M urea and 6 M guanidine hydrochloride) will not affect the purification properties of the media.

The data on chemical stability given in Table 1 refer to the results of tests where the chromatography media was exposed to different chemical agents for one week at 40°C. In no case was any significant change found in the chromatographic properties of the media.

Sephacryl HR also demonstrates notable thermal stability. It may be autoclaved repeatedly at 121°C, pH 7 for 30 min without affecting its chromatographic performance.

Note: Chromatograpy media should never be exposed to chemical or physical extremes for longer than absolutely necessary. Sephacryl HR media will withstand occasional short contact with 0.5 M NaOH provided it is washed with buffer or water immediately afterwards. A suitable cleaning procedure is described in the "Cleaning" section.

Sephacryl HR is normally used with aqueous eluents. However, due to the high stability of the matrix, it can also be used in organic solvents, such as acetone, ethanol, methanol, formamide, and dimethyl sulfoxide.

The chemical structure of Sephacryl HR renders the medium highly rigid as illustrated by the pressure/flow diagram in Figure 3.

Operation

Sephacryl HR is supplied ready-to-use in reproducible prepacked columns or as a suspension in 20% ethanol. The high rigidity and narrow particle size distribution of the chromatography medium ensures easy column packing. Complete packing and operating instructions are provided with each package.

Cleaning

When necessary, Sephacryl HR can be cleaned *in situ* with 1 to 2 bed volumes of 0.2 M NaOH or a nonionic detergent. After cleaning, ensure that the column is fully re-equilibrated with 2 to 3 bed volumes of buffer before re-using. We also recommend that 20% ethanol is used as a bacteriostatic storage solution when the column is not in use.

Characteristics of HiPrep columns

Sephacryl S-100 HR, S-200 HR, S-300 HR, S-400 HR, and S-500 HR are also available in two diameters of prepacked HiPrep columns. HiPrep 16/60 columns have an internal diameter of 16 mm and a bed height of 60 cm with a bed volume of approximately 120 ml. HiPrep 26/60 columns have an internal diameter of 26 mm and a bed height of 60 cm with a bed volume of approximately 320 ml.

HiPrep columns are made of polypropylene, which does not interact with biomolecules. The column is not designed to be opened or repacked.

The excellent purification characteristics of these prepacked columns are ensured by carefully testing the selectivity, nonspecific interaction, and particle size distribution of the chosen Sephacryl HR medium. The columns are then packed using validated packing procedures that include testing the column efficiency the number of theoretical plates per meter (N/m) for each production batch.

Table 2 summarizes the main characteristics of HiPrep Sephacryl HR, gel filtration columns.

Purification of standard proteins and dextrans

Figure 4 compares the purification of a mixture of standard proteins and dextrans on HiPrep 16/60 Sephacryl S-100 HR, S-200 HR, S-300 HR, S-400 HR, and S-500 HR columns.

Operation

HiPrep columns are simple to run with a single pump or with a chromatography system such as ÄKTAdesign.

Detailed instructions for running the columns are provided.

Cleaning

For optimal purification, we recommend checking the column performance at regular intervals. This is easily done and described in detail in the instructions available in the product package or visit www.gelifesciences.com/protein-purification.

Regular cleaning will prolong the long-term purification performance of HiPrep columns. Wash the column with one-half column volume of 0.2 M NaOH at 15 cm/h (0.5 ml/min HiPrep 16/60 or 1.3 ml/min HiPrep 26/60) to remove most proteins that are nonspecifically adsorbed to the medium.

Cleaning-in-place (CIP) procedures for removing protein and lipids bound more strongly to the medium are also included in instructions supplied with each column.

Table 2. Characteristics of HiPrep Sephacryl HR columns

Matrix	Cross-linked copolymer of allyl dextran and N,N′-methylene bisacrylamide	
Average particle size	47 µm	
Separation range	Globular proteins 1×10^3–1×10^5 5×10^3–2.5×10^5 1×10^4–1.5×10^6 2×10^4–8×10^6	 (Sephacryl S-100 HR) (Sephacryl S-200 HR) (Sephacryl S-300 HR) (Sephacryl S-400 HR)
	Dextrans 1×10^3–8×10^4 2×10^3–4×10^5 1×10^4–2×10^6 4×10^4–2×10^7	 (Sephacryl S-200 HR) (Sephacryl S-300 HR) (Sephacryl S-400 HR) (Sephacryl S-500 HR)
	HiPrep 16/60	**HiPrep 26/60**
Column volume	120 ml	320 ml
Sample volume*	Up to 5 ml	Up to 13 ml
Recommended flow rate†	0.5 ml/min (15 cm/h)	1.3 ml/min (15 cm/h)
Maximum flow rate†	1.0 ml/min (30 cm/h)	2.6 ml/min (30 cm/h)
Maximum pressure over the packed bed during operation	0.15 MPa, 1.5 bar	0.15 MPa, 1.5 bar
HiPrep column hardware pressure limit	0.5 MPa, 5 bar	0.5 MPa, 5 bar
Theoretical plates	> 5000 m^{-1}	> 5000 m^{-1}
pH stability Long term and working range Short term	 3–11 2–13	 3–11 2–13
Chemical stability	All commonly used aqueous buffers pH 3–11, 1 M acetic acid, 8 M urea, 6 M guanidine hydrochloride, 30% isopropanol, 30% acetonitrile, 20% ethanol, 0.5 M NaOH	
Storage	20% ethanol	20% ethanol

* Optimal sample volume depends on the complexity of the sample and the flow rate. If the sample contains substances with small differences in size, either decrease the sample volume, or decrease the flow rate. In very difficult cases, it may be necessary to decrease both

† 25°C in distilled water

Comparing the separation of standard proteins and dextrans

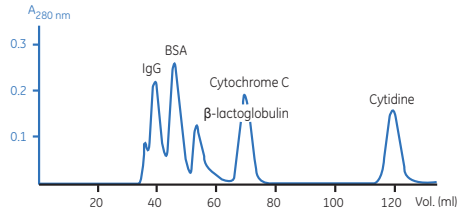
Column: a) HiPrep 16/60 Sephacryl S-100 HR
b) HiPrep 16/60 Sephacryl S-200 HR
c) HiPrep 16/60 Sephacryl S-300 HR

Sample: 500 µl of a mixture comprising IgG (M_r 160 000), BSA (M_r 67 000), β-lactoglobulin (M_r 35 000), cytochrome C (M_r 12 400), and cytidine (M_r 240)

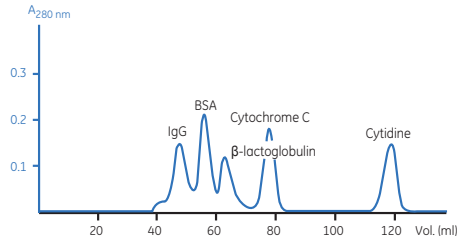
Buffer: 0.05 M sodium phosphate, 0.15 M NaCl, pH 7.0

Flow rate: 0.8 ml/min (24 cm/h)

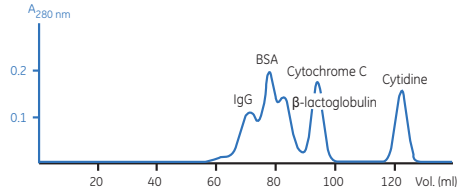
a) HiPrep 16/60 Sephacryl S-100 HR



b) HiPrep 16/60 Sephacryl S-200 HR



c) HiPrep 16/60 Sephacryl S-300 HR



Column: d) HiPrep 16/60 Sephacryl S-400 HR
e) HiPrep 16/60 Sephacryl S-500 HR

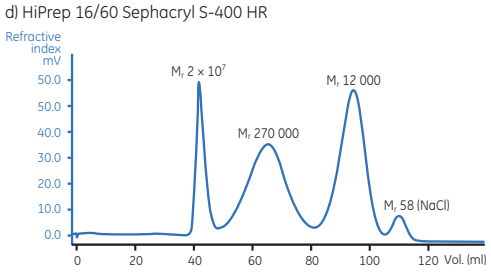
Sample: d) 1.2 ml of a sample containing three dextrans; $M_r > 20 \times 10^6$, M_r 270 000, and M_r 12 000

e) 1.2 ml of a sample containing three dextrans, $M_r > 20 \times 10^6$, M_r 1.8×10^6, and M_r 25 000

Buffer: 0.25 M NaCl

Flow rate: 0.5 ml/min (15 cm/h)

d) HiPrep 16/60 Sephacryl S-400 HR



e) HiPrep 16/60 Sephacryl S-500 HR

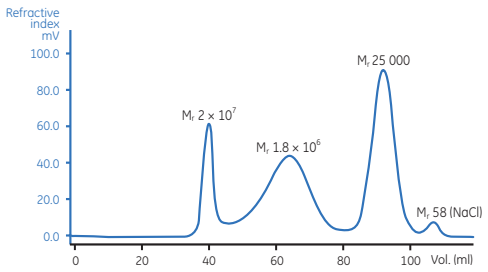


Fig 4. Comparison of the selectivity of the five different prepacked HiPrep Sephacryl HR columns.

Applications

Sephacryl HR media are well-proven and used for many applications by life scientists and industrial manufacturers all over the world.

Figures 5 to 10 show a number of laboratory-scale purification on the five Sephacryl HR media, including purifications performed on prepacked HiPrep Sephacryl HR columns. The industrial use of Sephacryl HR is described later.

Insulin consists of two chains (A and B) held together by -S-S- bonds. Figure 5 shows that when these links have been broken, the two chains can, despite small differences in molecular weight, be separated on HiPrep 26/60 Sephacryl S-100 HR.

Figure 6 shows the highly efficient purification of human growth hormone (hGH) dimers and monomers.

Purification of insulin chains

Column: HiPrep 26/60 Sephacryl S-100 HR

Sample: 1 ml of a mixture comprising bovine insulin chain A (M_r 2532) and chain B (M_r 3496), 0.5 mg/ml of each

Buffer: 0.05 M sodium phosphate, 0.15 M NaCl, pH 7.0

Flow rate: 2.0 ml/min (22 cm/h)

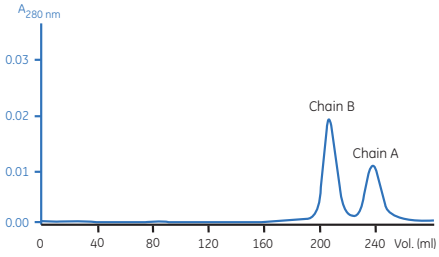


Fig 5. Purification of insulin chains on HiPrep Sephacryl 26/60 S-100 HR.

Purification of hGH dimers and monomers

Medium: Sephacryl S-100 HR packed bed, approx. 480 ml

Sample: 14 ml hGH solution, containing 6% dimers

Buffer: Glycine-phosphate, pH 7.0

Flow rate: 0.3 ml/min (4 cm/h)

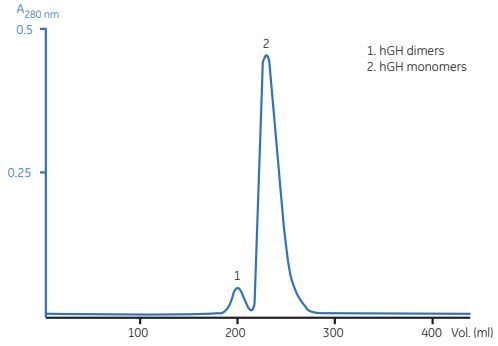


Fig 6. Human Growth Hormone (hGH) dimers and monomers are efficiently separated on Sephacryl S-100 HR.

Phytohemagglutinin M (PHA-M) is degraded in an acidic environment into an active protein and inactive polysaccharide. Figure 7 shows the purification of PHA-M and purified PHA-P on HiPrep 26/60 Sephacryl S-200 HR.

Purification of phytohemagglutinin

Column: HiPrep 26/60 Sephacryl S-200 HR
Sample: a) 1 ml PHA-M, 2 mg/ml in acetic acid
b) 1 ml PHA-P, 2 mg/ml in acetic acid
Buffer: 0.05 M sodium phosphate, 0.15 M NaCl, pH 7.0
Flow rate: 2.0 ml/min (22 cm/h)

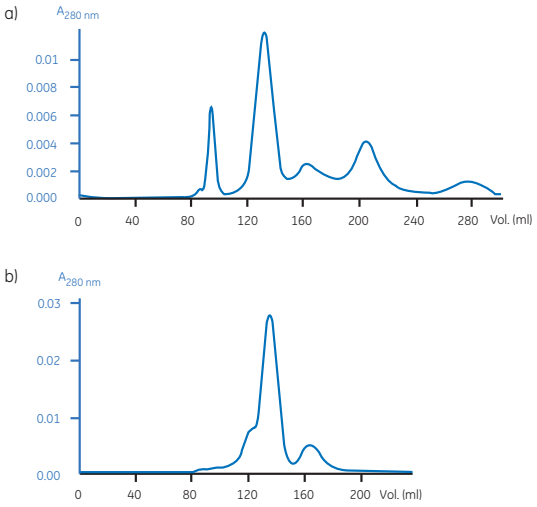


Fig 7. Purification of phytohemagglutinin on HiPrep 26/60 Sephacryl S-200 HR.

Purification of membrane proteins

Medium: Sephacryl S-300 HR packed in XK 26/70, approx. 340 ml
Sample: 500 µl of a mixture containing integral membrane proteins from human erythrocytes
Buffer: PBS, pH 7.0
Flow rate: 0.8 ml/min (9.4 cm/h)

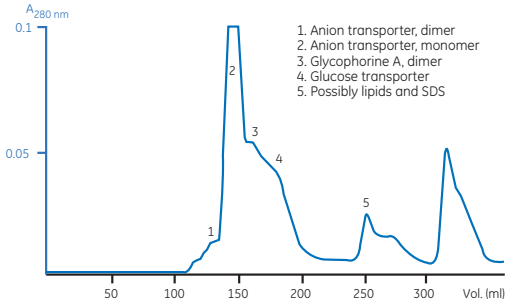


Fig 8. Elution profile of integral membrane proteins from human erythrocytes on Sephacryl S-300 HR. The proteins were resolved into four fractions (1–4), which contained mainly: dimer and monomer of anion transporter, dimer of glycophorin A, and glucose transporter. The last peak (5) is probably a mixture of lipids and SDS.

Purification of phospholipid vesicles

Medium: Sephacryl S-400 HR packed in XK 26/70 column, approx. 320 ml
Sample: 2 ml (2 mg/ml) Integral membrane proteins prepared from human erythrocytes solubilized in 0.1 M phosphate, 100 mM SDS, 1 mM EDTA, 1 mM DTE, pH 7.4
Buffer: 0.1 M phosphate, 50 mM SDS, 1 mM EDTA, 1 mM DTE, pH 7.4
Flow rate: 1 ml/min (11 cm/h)

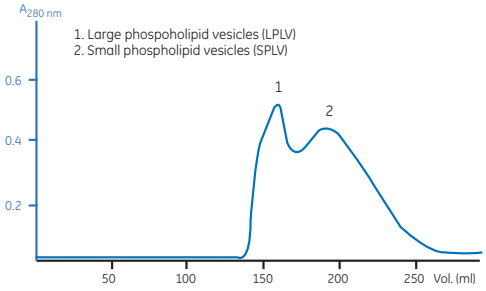


Fig 9. Gel filtration on Sephacryl S-400 HR can quickly purify phospholipid vesicles (liposomes) into large (LPLV) and small (SPLV) phospholipid vesicles.

Rapid purification of plasmids

Medium: Sephacryl S-500 HR packed in HR 16/10 column, bed size 1.6 × 10 cm
Sample: 1 ml DNA solution from *E. coli* HB 101 containing plasmid pRIT-18
Buffer: 0.1 M Tris-HCl, 0.1 M NaCl, 1 mM EDTA, pH 8.0
Flow rate: 3 ml/min (90 cm/h)

Preparation method

1. Culture *E. coli* HB 101 containing plasmid pRIT-18 at 37°C overnight in the presence of amplicin (70 mg/l) and Tryptic Soy Broth (30 g/l).
2. Centrifuge-cells (4000 × g, 5 min) and suspend in solution I*.
3. Add solution II* and incubate in a waterbath 10 min, 50°C, stirring every other minute.
4. Add solution III* and cool on ice for 5 min.
5. Centrifuge (15 000 × g, 5 min) and filter the supernatant through 0.45 µm or 0.22 µm filter.

* Solution I: 0.025 M Tris, 0.010 M EDTA, 1% glucose, pH 8.0
Solution II: 0.2 M NaOH, 1.5% SDS
Solution III: 3.0 M Na acetate, 2.0 M acetic acid

The volumes of solutions I, II, and III should be one fifth of the culture volume

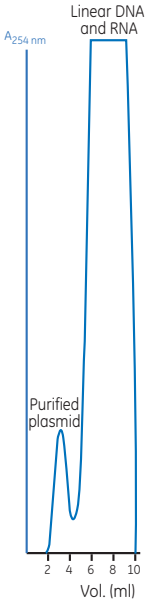


Fig 10. A rapid purification giving highly pure plasmid using Sephacryl S-500 HR.

Purification of virus-like particles

The virus-like particle (VLP) purified in this application contains the same proteins on the surface as a normal virus, but is unable to replicate and therefore poses no risk of infection. VLPs are used as vaccines and this type of vaccine offers great potential since they are likely to be highly immunogenic without the need for adjuvants.

To increase productivity of the purification process, it is important to determine the maximum amount of feed per milliliter of chromatography medium that can be loaded to give an acceptable level of purification. In this study, the effect of increased sample load was evaluated using the prepacked HiPrep 16/60 Sephacryl S-500 HR column.

The VLP sample had previously been purified on a strong anion exchange column, Capto™ Q, and concentrated by ultrafiltration/diafiltration.

The overlay of the gel filtration UV-absorbance at 280 nm showed a decrease in resolution with increased sample volume as expected (Fig 11a and peak enlarged in Fig 11b). The main peak was cut at the tailing side at approximately 1/3 of maximum peak height and the pool from the main peak was analyzed by SDS-PAGE, which revealed the increase in product purity compared to the start material (Fig 11c). However, it was not possible to determine the optimum amount of feed per milliliter chromatography medium. Other quantitative analyses would be needed to determine the level of impurities in the main peak.

Column: HiPrep 16/60 Sephacryl S-500 HR
Sample: Virus-like particle (VLP) in sodium phosphate buffer with NaCl, pH 7 (previously purified on a strong anion exchange column, Capto Q)
Sample load: 3%, 5%, 7%, and 9% of total column volume (3.6 ml, 6.0 ml, 8.4 ml, and 10.8 ml)
Buffer: 25 mM sodium phosphate, 500 mM NaCl, pH 7.2
Flow rate: 1 ml/min (30 cm/h)
System: ÄKTAexplorer™ 100

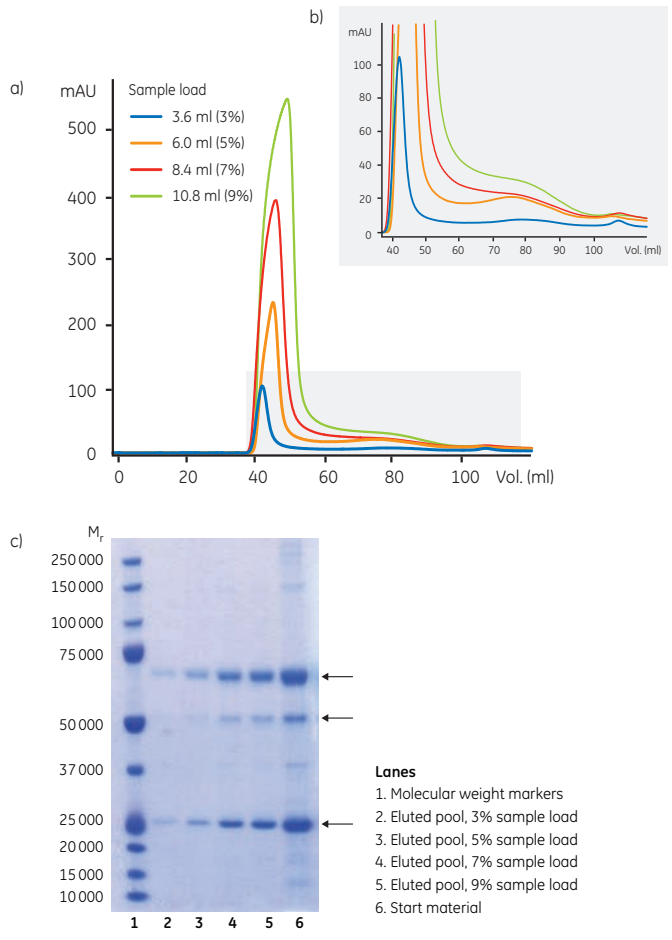


Fig 11. a). Purification of a virus-like particle (VLP) by gel filtration using HiPrep 16/60 Sephacryl S-500 HR. Various sample volumes were loaded on the column. b). Enlargement of peaks presented in a). c). SDS-PAGE analysis (reducing conditions, 4–12 % polyacrylamide gel, Coomassie™ stained) of eluted pools where the arrows indicate surface proteins of the VLP (M_r 69 000, 54 000, and 27 000).

Industrial use of Sephacryl HR media

The excellent resolution and high chemical stability of Sephacryl High Resolution enables the use of the chromatography medium in industrial-scale applications. Sephacryl S-200 HR in particular is well established for use in a number of large-scale applications, especially in the field of serum protein purification. For example, the high resolution of Sephacryl S-200 HR has been used to optimize a polymer removal step in a major albumin production process. Here, no less than three production batches are now processed in a single column volume by successive sample additions (Fig 12). The productivity of the process increased three-fold.

Repeated cycles of albumin fractionation

Column: BP 113/120 packed with Sephacryl S-200 HR, bed height 100 cm
Sample: Albumin fraction from previous ion exchange step. Sample load equivalent to 4% of V_t (V_t = column volume). Sample concentration 15 mg/ml
Buffer: 0.05 M Tris-HCl, 0.15 M NaCl, pH 7.5
Flow rate: 7.5 cm/h

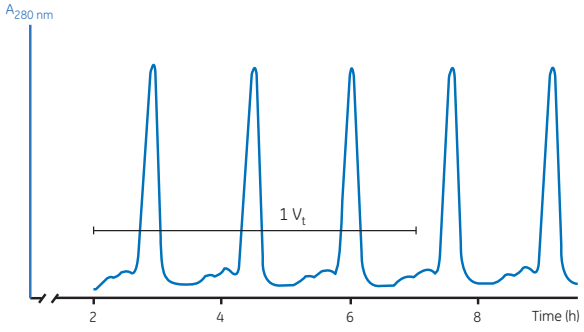


Fig 12. Repeated cycles of gel filtration using Sephacryl S-200 HR of albumin obtained from a previous ion exchange chromatography step. The sample peaks are spaced so that three purifications can be performed on the same column simultaneously, within an elution volume of V_t. The time equivalent to the passage of V_t is shown.

Storage

All Sephacryl HR chromatography media and HiPrep Sephacryl HR prepacked columns should be stored in 20% ethanol at 4°C to 30°C.

Acknowledgement

Virus-like particle (VLP) sample (see data in Fig 11) was provided by Novavax, Inc., Belward Campus Drive, Rockville, MD 20850, USA. We thank Novavax for fruitful discussions and excellent collaboration.

Data in Figs 8 and 9 were kindly provided by E. Greijer and P. Lundahl, Dept. of Biochemistry, Biomedical Centre, University of Uppsala, Sweden.

Data in Fig 10 was kindly provided by T. Moks, Department of Biochemistry, Royal Institute of Technology, Stockholm, Sweden.

Ordering information

Product	Quantity	Code No.
Sephacryl S-100 HR	150 ml	17-0612-10
	750 ml	17-0612-01
Sephacryl S-200 HR	150 ml	17-0584-10
	750 ml	17-0584-01
Sephacryl S-300 HR	150 ml	17-0599-10
	750 ml	17-0599-01
Sephacryl S-400 HR	150 ml	17-0609-10
	750 ml	17-0609-01
Sephacryl S-500 HR	150 ml	17-0613-10
	750 ml	17-0613-01

Product	Quantity	Code No.
HiPrep 16/60 Sephacryl S-100 HR	1 × 120 ml	17-1165-01
HiPrep 26/60 Sephacryl S-100 HR	1 × 320 ml	17-1194-01
HiPrep 16/60 Sephacryl S-200 HR	1 × 120 ml	17-1166-01
HiPrep 26/60 Sephacryl S-200 HR	1 × 320 ml	17-1195-01
HiPrep 16/60 Sephacryl S-300 HR	1 × 120 ml	17-1167-01
HiPrep 26/60 Sephacryl S-300 HR	1 × 320 ml	17-1196-01
HiPrep 16/60 Sephacryl S-400 HR	1 × 120 ml	28-9356-04
HiPrep 26/60 Sephacryl S-400 HR	1 × 320 ml	28-9356-05
HiPrep 16/60 Sephacryl S-500 HR	1 × 120 ml	28-9356-06
HiPrep 26/60 Sephacryl S-500 HR	1 × 320 ml	28-9356-07

Accessories	Quantity	Code No.
HiTrap™/HiPrep 1/16" male connector to ÄKTAdesign	8	28-4010-81
Union M6 female/1/16" male* (for connection to FPLC™ Systems)	5	18-3858-01

* Two unions (in red polypropylene) are included in HiPrep package

Related literature	Quantity	Code No.
Gel Filtration Handbook, Principles and Methods	1	18-1022-18
Gel Filtration Column and Media, Selection Guide	1	18-1124-19
Prepacked chromatography columns for ÄKTAdesign and Ettan™ LC systems, Selection guide	1	28-9317-78

For contact information for your local office, please visit, www.gelifesciences.com/contact

www.gelifesciences.com/protein-purification

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

imagination at work

GE Healthcare UK Limited
Amersham Place
Little Chalfont
Buckinghamshire, HP7 9NA
UK

GE Healthcare Europe, GmbH
Munzinger Strasse 5
D-79111 Freiburg
Germany

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327
Piscataway, NJ 08855-1327
USA

GE Healthcare Bio-Sciences KK
Sanken Bldg., 3-25-1, Hyakunincho
Shinjuku-ku, Tokyo 169-0073
Japan

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