Sephacryl High Resolution resins
HiPrep Sephacryl HR columns

SIZE EXCLUSION CHROMATOGRAPHY

Sephacryl™ High Resolution (HR) chromatography resins allow fast and reproducible purification of proteins, polysaccharides, and other macromolecules by size exclusion chromatography (SEC) at laboratory and industrial scale. Five Sephacryl HR chromatography resins 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 resins include:

- Purification over a wide molecular weight range
- High reproducibility due to high stability
- High recoveries
- Well-suited to industrial-scale use

All five Sephacryl HR resins are available in prepacked HiPrep™ Sephacryl HR SEC columns. Each chromatography resin is available in two different prepacked column sizes; 16/60 (120 mL) and 26/60 (320 mL). HiPrep Sephacryl HR SEC columns provide the excellent purification properties of Sephacryl HR resins in a convenient, ready-to-use format.

Characteristics of the columns include:

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

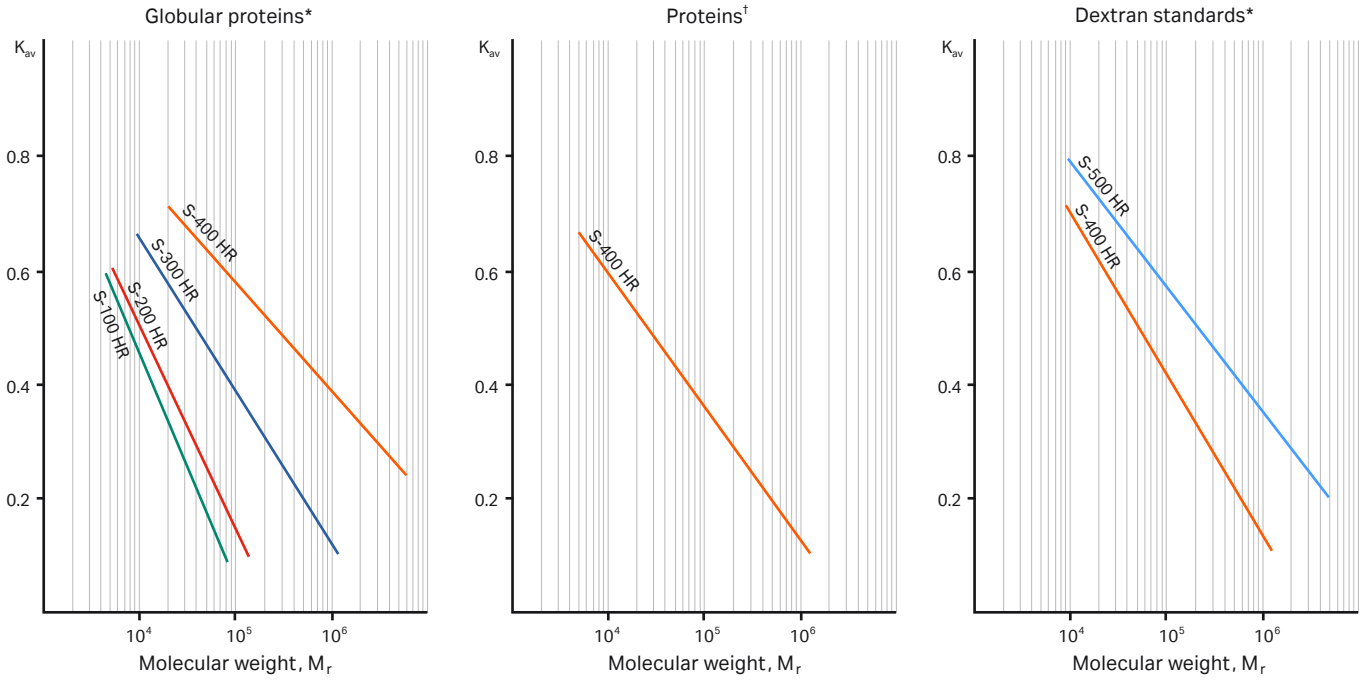
Fig 1. Sephacryl HR chromatography resins and HiPrep Sephacryl HR prepacked columns offer the user a wide range of choice and reliable purification by SEC.

Characteristics of resins

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

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

Sephacryl HR SEC resins fulfill process chromatography requirements in terms of stability, scalability, and bulk availability. As members of the BioProcess™ family of chromatography resins, 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 resins.

The five chromatography resins 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 characteristics of Sephacryl HR resins.

Sephacryl HR resins can be packed in most column types, including wide diameter production columns with bed heights of 60 to 100 cm.

Table 1. Characteristics of Sephacryl HR resins

Sephacryl	S-100 HR	S-200 HR	S-300 HR	S-400 HR	S-500 HR
Fractionation 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
Exclusion limit DNA (base pairs)	–	~ 30 bp	~ 118 bp	~ 271 bp	~ 1078 bp
Particle size, d_{50v} [1]	~ 50 µm	~ 50 µm	~ 50 µm	~ 50 µm	~ 50 µm
Matrix	Cross-linked copolymer of allyl dextran and N,N'-methylene bisacrylamide				
Chemical stability	Stable to commonly used aqueous buffers: 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				
pH stability, operational[2]	3–11	3–11	3–11	3–11	3–11
pH stability, CIP[3]	2–13	2–13	2–13	2–13	2–13
Physical stability	Negligible volume variation due to changes in pH or ionic strength				
Autoclavability	20 min at 121°C in 0.15 M NaCl pH 7, 5 cycles (resin only)				
Storage	20% ethanol, 4°C to 30°C	20% ethanol, 4°C to 30°C	20% ethanol, 4°C to 30°C	20% ethanol, 4°C to 30°C	20% ethanol, 4°C to 30°C
Pressure/flow characteristics	≥ 125 cm/h[4,5]	≥ 150 cm/h[4,5]	≥ 150 cm/h[4,5]	≥ 150 cm/h[4,5]	≥ 125 cm/h[4,5]

[1] *Median particle size of the cumulative volume distribution.*
[2] *pH range where resin can be operated without significant change in function.*
[3] *pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.*
[4] *At < 0.1 MPa in a XK 50/30 column with 5 cm diameter and 15 cm bed height (at 20°C using buffers with a viscosity similar to water).*
[5] *The pressure/flow characteristics describes the relationship between pressure and flow under the set circumstances. The pressure given shall not be taken as the maximum pressure of the resin.*

Stability

Sephacryl HR is highly stable, both chemically and physically. The chromatography resin is compatible with aqueous buffers (from pH 3 to 11) commonly used in biochemistry, and withstands strong bases (e.g., 0.5 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 resins.

The data on chemical stability given in Table 1 refer to the results of tests where the chromatography resins were 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 resins.

Sephacryl HR also demonstrates notable thermal stability. It may be autoclaved repeatedly at 121°C, pH 7 for 20 min without affecting its chromatographic performance.

Note: Chromatograpy resins should never be exposed to chemical or physical extremes for longer than absolutely necessary. Sephacryl HR resins 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.

Operation

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

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

Table 2 summarizes the main characteristics of HiPrep Sephacryl HR SEC columns.

Purification of standard proteins and dextrans

Figure 3 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 an ÄKTA system.

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 cytiva.com/protein-purification.

Regular cleaning will prolong the long-term purification performance of HiPrep columns. Wash the column with a half-column volume of 0.5 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 resin.

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

Table 2. Characteristics of HiPrep Sephacryl HR columns

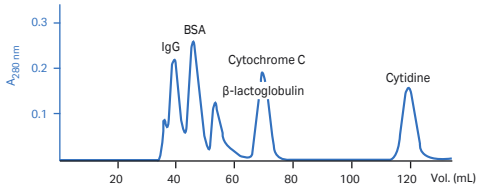
	HiPrep 16/60	HiPrep 26/60
Column volume	120 mL	320 mL
Sample volume[1]	Up to 5 mL	Up to 13 mL
Recommended operating flow rate[2]	0.5 mL/min (15 cm/h)	1.3 mL/min (15 cm/h)
Maximum operating flow rate[2]	1.0 mL/min (30 cm/h)	2.6 mL/min (30 cm/h)
Maximum operating pressure	0.15 MPa (1.5 bar, 21.7 psi)	0.15 MPa (1.5 bar, 21.7 psi)
HiPrep column hardware pressure limit	0.5 MPa (5 bar, 72.5 psi)	0.5 MPa (5 bar, 72.5 psi)
Theoretical plates	> 5000 m^{-1}	> 5000 m^{-1}
pH stability, operational[3]	3–11	3–11
pH stability, CIP[4]	2–13	2–13
Chemical stability	Stable to commonly used aqueous buffers: pH 3–11, 2 M NaCl, 1 M acetic acid, 8 M urea, 6 M guanidine hydrochloride, 30% isopropanol, 30% acetonitrile, 20% ethanol	
Storage	20% ethanol, 4°C to 30°C	20% ethanol, 4°C to 30°C

[1] 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.
[2] At room temperature in distilled water.
[3] pH range where resin can be operated without significant change in function.
[4] pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

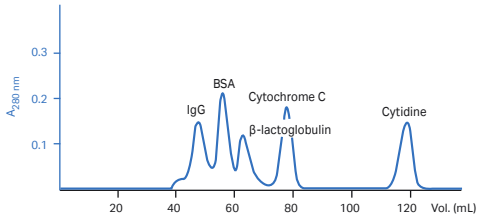
Comparing the separation of standard proteins and dextrans

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)

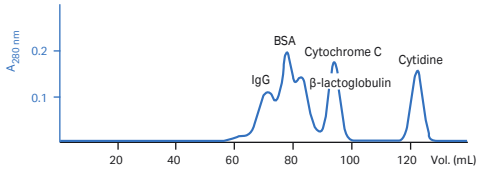
(A) HiPrep 16/60 Sephacryl S-100 HR



(B) HiPrep 16/60 Sephacryl S-200 HR



(C) HiPrep 16/60 Sephacryl S-300 HR

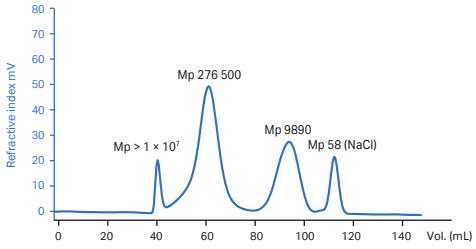


Column: (D) HiPrep 16/60 Sephacryl S-400 HR
(E) HiPrep 16/60 Sephacryl S-500 HR
Sample: (D) 1 mL of a sample containing a mixture of
1. high molecular weight fraction of Dextran >1 × 10^7 (Cytica) fractionated from raw dextran,
2. Dextran 410 (M^{peak} 276 500, Pharmacosmos), and
3. Dextran 12 (M^{peak} 9890, Pharmacosmos)

(E) Mixture of
1. High molecular weight fraction of Dextran >1 × 10^7 (Cytiva) fractionated from raw dextran,
2. Dextran DXT1185K (M^{peak} 1.05 × 10^6 American Polymer Standards Corp), and
3. Dextran 50 (M^{peak} 43 500, Pharmacosmos)

Buffer: 0.25 M NaCl
Flow rate: 0.5 mL/min (15 cm/h)
Detection: Refractive index (RI)

(D) HiPrep 16/60 Sephacryl S-400 HR



(E) HiPrep 16/60 Sephacryl S-500 HR

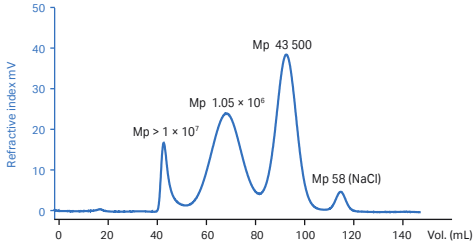


Fig 3. Comparison of the selectivity of the five different prepacked HiPrep Sephacryl HR columns.

Applications

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

Figures 4 to 10 show a number of laboratory-scale purifications on the five Sephacryl HR resins, including purifications performed on prepacked HiPrep Sephacryl HR columns. The industrial use of Sephacryl HR is described later.

Purification of insulin chains

Insulin consists of two chains (A and B) held together by -S-S bonds. Figure 4 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.

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)

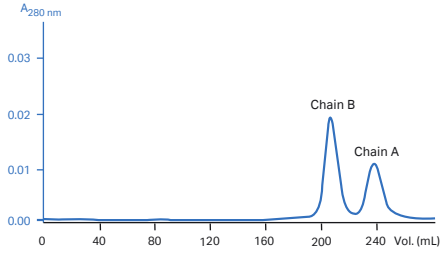


Fig 4. Purification of insulin chains on HiPrep Sephacryl 26/60 S-100 HR.

Purification of hGH dimers and monomers

Figure 5 shows the purification of human growth hormone (hGH) dimers and monomers.

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)

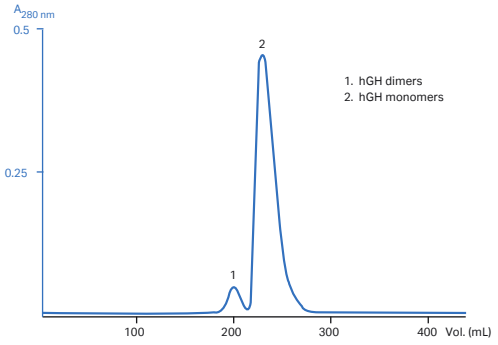


Fig 5. Human Growth Hormone (hGH) dimers and monomers are efficiently separated on Sephacryl S-100 HR.

Purification of phytohemagglutinin

Phytohemagglutinin M (PHA-M) is degraded in an acidic environment into an active protein and inactive polysaccharide. Figure 6 shows the purification of PHA-M and purified PHA-P on HiPrep 26/60 Sephacryl S-200 HR.

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)

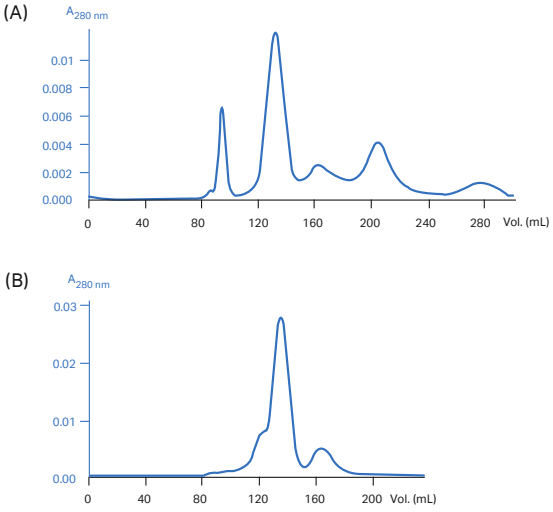


Fig 6. Purification of phytohemagglutinin on HiPrep 26/60 Sephacryl S-200 HR.

Purification of membrane proteins

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.

Column:	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)

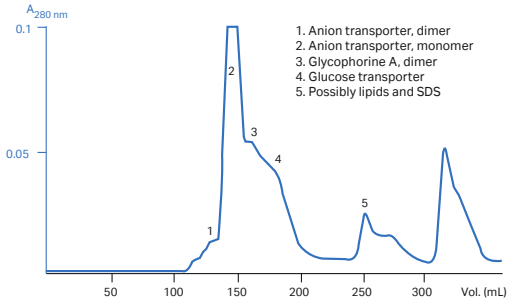


Fig 7. Elution profile of integral membrane proteins from human erythrocytes on Sephacryl S-300 HR.

Purification of phospholipid vesicles

Column:	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)

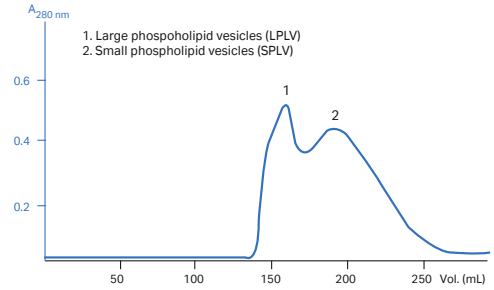


Fig 8. Fractionation of phospholipid vesicles (liposomes) into large (LPLV) and small (SPLV) sizes using Sephacryl S-400 HR.

Rapid purification of plasmids

Preparation was performed according to the following procedure:

1. Culture *E. coli* HB 101 containing plasmid pRIT-18 at 37°C overnight in the presence of ampicillin (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

Resin: 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)

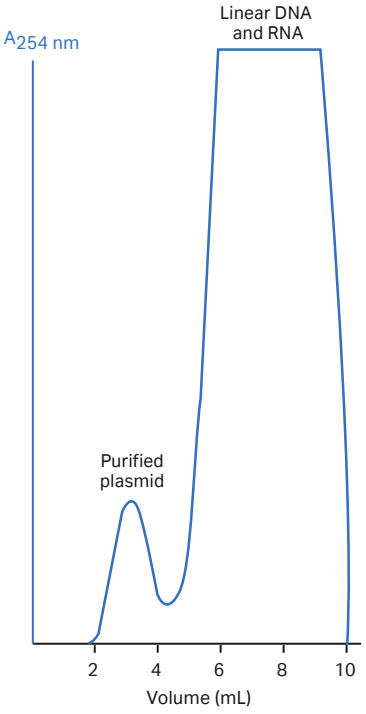


Fig 9. A rapid purification giving highly pure plasmid using Sephacryl S-500 HR.

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 it is 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 resin 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 SEC UV-absorbance at 280 nm showed a decrease in resolution with increased sample volume as expected (Fig 10A and peak enlarged in Fig 10B). 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 10C).

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)

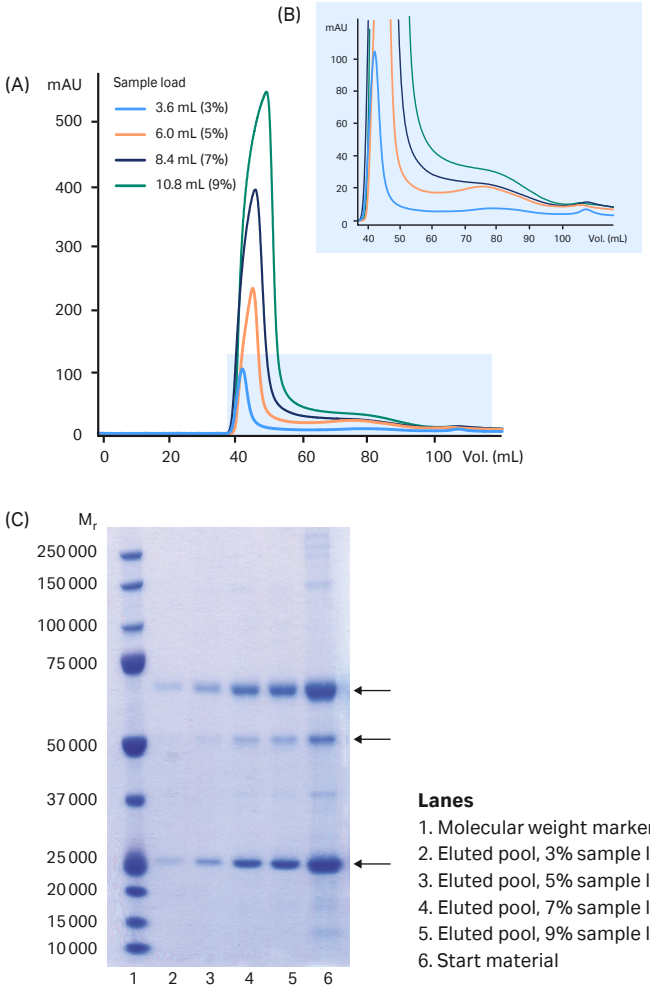


Fig 10. (A). Purification of a virus-like particle (VLP) by SEC using HiPrep 16/60 Sephacryl S-500 HR. Various sample volumes were loaded on the column. (B) Enlargement of peaks presented in (A) (C) SDS-PAGE analysis (reducing conditions, 4–12 % polyacrylamide gel, Coomassie™ stained) of eluted pools where the arrows indicate surface proteins of the VLP (M_r 69 000, 54 000, and 27 000).

Industrial use of Sephacryl HR resins

The high resolution and chemical stability of Sephacryl HR enables the use of the chromatography resin in industrial-scale applications. Here is shown the example, of Sephacryl S-200 HR being 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 11). 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 velocity: 7.5 cm/h

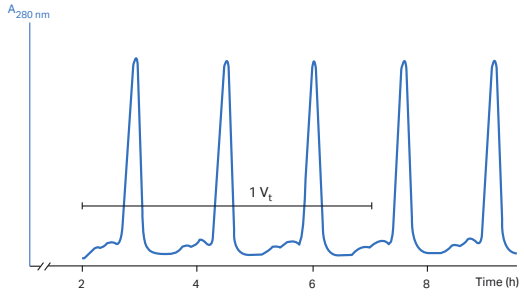


Fig 11. Repeated cycles of SEC using Sephacryl S-200 HR of albumin obtained from a previous ion exchange chromatography step. The sample peaks are spaced so that three purifications can be performed on the same column simultaneously, within an elution volume of V_t. The time equivalent to the passage of V_t is shown.

Storage

All Sephacryl HR chromatography resins 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 10) was provided by Novavax, Inc., Belward Campus Drive, Rockville, MD 20850, USA. We thank Novavax for fruitful discussions and excellent collaboration.

Data in Figs 7 and 8 were kindly provided by E. Greijer and P. Lundahl, Dept. of Biochemistry, Biomedical Centre, University of Uppsala, Sweden.

Data in Fig 9 was kindly provided by T. Moks, Department of Biochemistry, Royal Institute of Technology, Stockholm, Sweden.

Ordering information

Product	Quantity	Product code
Sephacryl S-100 HR	150 mL	17061210
	750 mL	17061201
	10 L	17061205
Sephacryl S-200 HR	150 mL	17058410
	750 mL	17058401
	10 L	17058405
Sephacryl S-300 HR	150 mL	17059910
	750 mL	17059901
	10 L	17059905
Sephacryl S-400 HR	150 mL	17060910
	750 mL	17060901
	10 L	17060905
Sephacryl S-500 HR	150 mL	17061310
	750 mL	17061301
	10 L	17061305
HiPrep 16/60 Sephacryl S-100 HR	1 × 120 mL	17116501
HiPrep 26/60 Sephacryl S-100 HR	1 × 320 mL	17119401
HiPrep 16/60 Sephacryl S-200 HR	1 × 120 mL	17116601
HiPrep 26/60 Sephacryl S-200 HR	1 × 320 mL	17119501
HiPrep 16/60 Sephacryl S-300 HR	1 × 120 mL	17116701
HiPrep 26/60 Sephacryl S-300 HR	1 × 320 mL	17119601
HiPrep 16/60 Sephacryl S-400 HR	1 × 120 mL	28935604
HiPrep 26/60 Sephacryl S-400 HR	1 × 320 mL	28935605
HiPrep 16/60 Sephacryl S-500 HR	1 × 120 mL	28935606
HiPrep 26/60 Sephacryl S-500 HR	1 × 320 mL	28935607

Accessories

HiTrap™/HiPrep 1/16" male connector for ÄKTA system*	8	28401081
Union M6 female/1/16" male	5	18385801

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

Related Literature

Size Exclusion Chromatography Handbook, Principles and Methods	1	18102218
Size exclusion chromatography columns and media, Selection guide	1	18112419
Prepacked chromatography columns for ÄKTA systems, Selection guide	1	28931778

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