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Size exclusion chromatography columns and media

Selection guide

Distributor
GE Healthcare

General information

Principles of size exclusion chromatography

Size exclusion chromatography (SEC), also called gel filtration (GF), separates molecules on the basis of differences in size as they pass through a SEC medium packed in a column. SEC media consist of spherical particles with pores of different sizes where molecules small enough to enter the pores are retarded as compared to larger molecules (Fig 1). Samples are eluted isocratically (single buffer, no gradient). Buffer conditions can be varied to suit the sample type or the requirements for the next purification, analysis, or storage step.

A variety of SEC media with different selectivities are available and cover a molecular weight range (M_r) from M_r 100 to 100 000 000, from peptides to very large proteins, protein complexes, and viruses.

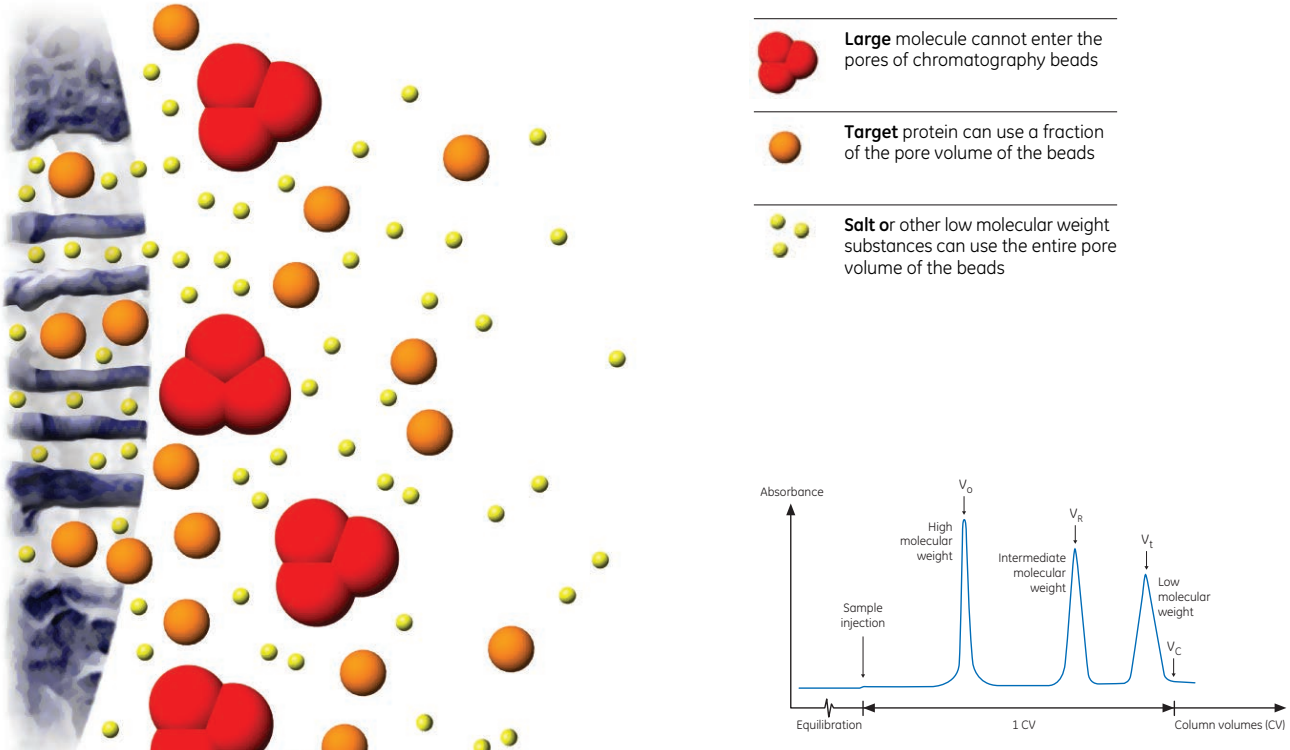


Fig 1. Schematic process of SEC.

SEC can be applied in two ways

1. **Group separations** where the components of a sample are separated into two major groups according to size range (Fig 2). A group separation can be used to remove high or low molecular weight contaminants, such as phenol red from culture fluids, or for desalting and buffer exchange.
2. **High-resolution fractionation** of biomolecules where the components of a sample are separated according to differences in their molecular size (Fig 3). High-resolution fractionation can be used to isolate one or more components, to separate monomers from aggregates, or to perform a molecular weight distribution analysis. High-resolution SEC is most suitable for samples that originally contain few components or for samples that have been partially purified by other chromatography techniques so that most of the unwanted proteins of similar size are eliminated.

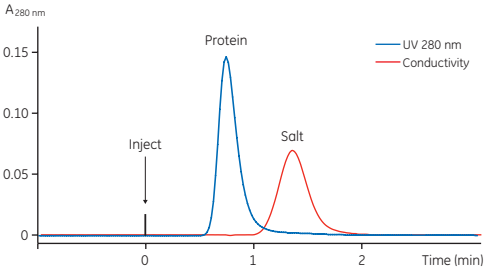


Fig 2. Typical group separation.

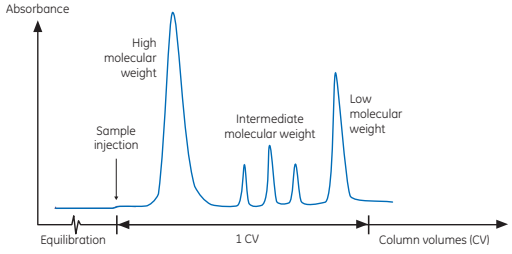


Fig 3. Typical high-resolution SEC separation.

Chromatography media selection

Group separation

Sephadex™ is excellent for rapid group separations such as desalting and buffer exchange, before, between, or after other chromatography purification. This SEC medium can be used at both laboratory and production scale.

High-resolution fractionation

Superdex™ is the first choice for high-resolution fractionation, short run times, and high recovery.

Sephacryl™ is suitable for fast, high recovery separations at laboratory and industrial scale.

Superose™ offers a broad fractionation range, but is not suitable for large scale or industrial-scale separations.

Note: The highest resolution is obtained with the new generation SEC media: Superdex Increase and Superose Increase.

Rapid purity check and screening

Superdex 75 Increase 5/150 GL, Superdex 200 Increase 5/150 GL and Superose 6 Increase 5/150 GL are short columns with small bed volumes that are suitable for rapid protein homogeneity analyses or purity checks. They save time when screening many samples, and require less buffer and sample than longer columns.

Practical considerations

Selection of SEC media

Resolution is a function of the selectivity of the SEC medium, that is the distance between two peaks, and the efficiency of the medium, that is the ability to produce narrow peaks. The fractionation range defines the **range of molecular weights** that have access to the pores of the matrix; molecules within this range can be separated by high-resolution fractionation. The **exclusion limit** for an SEC medium indicates the size of the molecules that are excluded from the pores of the matrix and therefore elute in the void volume.

The selectivity of a SEC medium depends on its pore size distribution and is described by a selectivity curve (Fig 4). The steeper the selectivity curve, the higher the resolution that can be achieved. Resolution is also affected by band-broadening, which is dependent on the bead size of the SEC medium. The smaller the bead size, the higher the resolution.

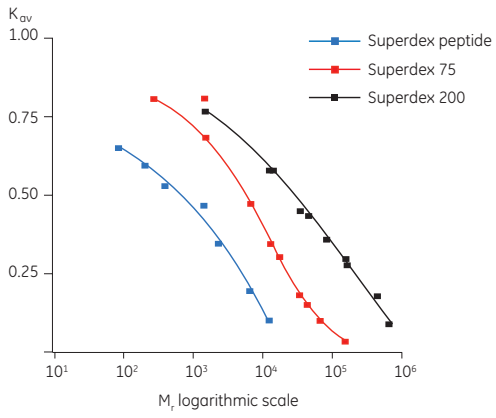


Fig 4. Selectivity curves for Superdex.

In cases where two SEC media have a similar fractionation range, select the SEC medium with the steepest selectivity curve for best resolution of all components in the sample. When you are interested in a specific component, select the medium where the target protein falls in the middle of the selectivity curve.

The success of SEC depends primarily on choosing conditions that give sufficient selectivity and counteract peak broadening effects during the separation. After the selection of an SEC medium, sample volume and column dimensions are the two most critical parameters that affect the resolution of the separation.

Bead size

For a given column dimension, the resolution can be improved by using smaller bead size. However, using a smaller bead size can increase in back pressure so that flow rate must be decreased and run time extended.

Column dimensions

The **height of the packed bed** affects both resolution and the time taken for elution. The resolution in SEC increases with the square root of bed height. Doubling the bed height gives an increase in resolution equivalent to $\sqrt{2} = 1.4$ (40%). For high resolution and fractionation, long columns will give the best results. The effective bed height can be increased by coupling columns containing the same SEC media in series.

For maximum resolution, **the dead volume should be kept at a minimum**; short, narrow capillaries should be used and unnecessary system components should be bypassed. This is especially important for micro preparative and analytical applications.

Sample and buffer preparation

Removal of particles in the sample is extremely important for SEC. Clarifying a sample by centrifugation and/or filtration before application onto a column will avoid the risk of blockage, reduce the need for stringent washing procedures, and extend the life of the SEC medium.

Buffer composition will generally not directly influence the resolution unless the buffer affects the shape or biological activity of the molecules. Select buffer conditions that are compatible with protein stability and activity and include between 25 and 150 mM NaCl to avoid nonspecific ionic interactions with the matrix which can result in delays in peak elution and poor reproducibility.

Always use high quality water and chemicals and filter all solutions through 0.45µm or 0.22 µm filters before use.

Whatman™ filters, which give the least amount of nonspecific binding of proteins, are composed of cellulose acetate (CA), regenerated cellulose (RA), or polyvinylidene fluoride (PVDF) membranes. To learn more about our range, visit www.gelifesciences.com/LabFiltration.

Sample volume

Smaller **sample volumes** help to avoid overlap between closely spaced peaks. For high-resolution fractionation, a sample volume from 0.5% to 4% of the total column volume (CV) is recommended, depending on the type of SEC medium used. For most applications the sample volume should not exceed 2% to achieve maximum resolution. For group separations, use sample volumes up to 30% of the total CV.

Flow rate

The resolution depends on the flow rate for mainly two reasons: A flow rate that is too high gives insufficient time for the molecules to equilibrate between the beads and the elution buffer, while a flow rate that is too low gives broadening of the peaks as a result of diffusion. The practical optimum for proteins is often in the range of 2 to 10 cm/h.

Note that lower flow rate should be used for high viscosity solutions and low temperature (2°C to 8°C).

Viscosity

High sample viscosity causes instability of the separation and an irregular flow pattern, leading to very broad and skewed peaks. To increase the capacity of a SEC separation, the sample may need to be concentrated. Note that the solubility or the viscosity of the sample can limit the concentration that can be used.

Transport device

Prepacked SEC columns are delivered with a storage/shipping device that keeps the pressure in the column and thereby prevents it from drying out. We recommend that you connect the storage/shipping device according to instructions supplied with the column for long-term storage.

Setting column pressure limits

To protect the column hardware and the packed bed of the chromatographic medium, it is important to set limits that must not be exceeded during the run. There are two important pressure limits that must be taken into consideration:

1. **To protect the column hardware:** Column hardware pressure limit, which is the maximum pressure the hardware can withstand without being damaged. This value is fixed for each column type. Leakage from the column could be a sign of excessive pressure on the column hardware. The column hardware pressure limit is included in the instructions and in UNICORN™ column list for each column type, respectively.
2. **To protect the packed bed.**

A value for maximum pressure or typical pressure drop over the packed bed (Δp) is given to protect the packed bed from compression; do not exceed this value at any time. For columns having a given typical pressure value, we recommend that you determine the individual column pressure limit according to the procedure described in the instruction (see for example Instructions 29027271). The packed bed is best protected by controlling the flow rate. Use lower flow rates for high-viscosity solutions and/or low temperature.

Column efficiency test

GE Healthcare packs columns to the highest standards, and each column is thoroughly tested with regard to the number of theoretical plates (Fig 5).

Column performance should be checked at regular intervals to determine column efficiency and peak symmetry, either by injecting acetone or by running a set of standard proteins relevant for the application used. Note that the result for column efficiency is dependent on the system used, including the capillaries and dead volumes. This means that the column efficiency given in the specification for the column (tested on another system) will not be exactly the same as your initial column efficiency result.

Sample: Acetone 20 mg/ml
Sample volume: 0.2% of the total packed column volume
Eluent: Distilled water
Flow rate: see recommended flow rate in the Instructions for the column
Temperature: Room temperature (25°C)

Column efficiency is calculated using the equation:

$N/m = 5.54 \times (V_R/w_h)^2/L$

where

V_R = Peak retention (elution) volume, w_h = Peak width at half peak height, V_R and w_h given in same units, L = bed height (m)

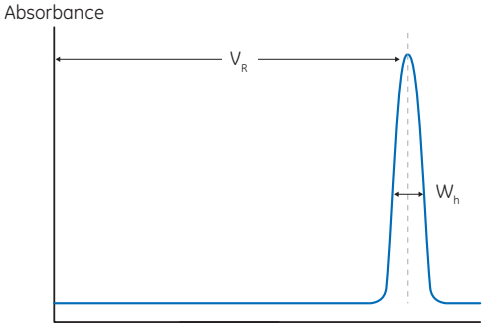


Fig 5. Column efficiency test.

Optimization

Perform a first run as described in the enclosed Instructions for the column. If the results obtained are unsatisfactory, consider the following:

Action	Effect
Decrease flow rate	Improved resolution for high molecular weight biomolecules The resolution for small biomolecules can decrease
Decrease sample volume	Improved resolution

Maintenance

Note: The description of regular cleaning below refers to Superdex and Superose columns; for other SEC media please read the respective instruction.

Regular cleaning

Perform the following regular cleaning cycle after every 10 to 20 separation cycles.

Wash the column with 0.5 to 1 CV of 0.5 M NaOH at a low flow rate to remove most nonspecifically adsorbed proteins. Wash with 2 CV of distilled water. Re-equilibrate the column with at least 2 CV of buffer. Further equilibration is necessary if your buffer contains detergent. Wait until the UV baseline stabilizes before applying next sample. Note that the column should never be stored in sodium hydroxide.

More rigorous cleaning

If cleaning using sodium hydroxide is not sufficient, additional cleaning using for example 30% isopropanol can be useful. Check the instruction for your specific column on details of the cleaning procedure.

As an alternative to more rigorous cleaning or if the column performance is still not restored, replace the filter at the top of the column, contaminants introduced with the liquid flow can be caught by the filter. After replacement of the filter, clean the column according to “Regular cleaning”. See also Procedure 29140760 for maintenance and cleaning of SEC columns.

Storage

If the column is to be stored more than two days after use, wash the column with 2 CV of distilled water, and then equilibrate with at least 2 CV of 20% ethanol (for HiLoad Superdex 30 pg and Superdex 75 pg, use 200 mM sodium acetate in 20% ethanol).

Note: Use a lower flow rate for viscous 20% ethanol.

Flow rate conversion

Flow rate is measured in volume terms, for example ml/min, but when comparing results between columns of different sizes it is useful to use the linear flow velocity, cm/h. To convert between linear flow velocity and volumetric flow rate, use the following formulas:

From linear flow velocity (cm/h) to volumetric flow rate (ml/min)

$$\text{Volumetric flow rate (ml/min)} = \frac{\text{Linear flow velocity (cm/h)}}{60} \times \text{column cross-sectional area (cm}^2\text{)}$$

From volumetric flow rate (ml/min) to linear flow velocity (cm/h)

$$\text{Linear flow velocity (cm/h)} = \frac{\text{Volumetric flow rate (ml/min)} \times 60}{\text{column cross-sectional area (cm}^2\text{)}}$$

For more information, please refer to the handbook *Size Exclusion Chromatography, Principles and Methods*, which can be ordered from GE Healthcare or downloaded at www.gelifesciences.com/handbooks.

Figure 6 summarizes which column to choose in terms of scale of purification, sample volume, and desired resolution.

Troubleshooting

Symptom	Remedy
Increased back pressure	Clean the column according to the section "Maintenance"
Loss of resolution and/or decreased sample recovery	Clean the column according to the section "Maintenance"
Air in the column	Reverse flow direction and pump 5 CV of well degassed water through the column at a low flow rate
Space between adapter and SEC medium	Stop the flow. Close the outlet tubing with the domed nut and then disconnect the inlet tubing. Adjust the adapter to the SEC medium surface according to instructions for the specific column. Reconnect the inlet tubing immediately avoiding to get air into the column. Note that some prepacked columns cannot be opened (e.g HiPrep and Precision columns).
Low resolution	Minimize dead volumes in the chromatographiy system by decreasing the capillary length between the injector and the detector. You can also change to capillaries with smaller diameter given even less dead volume but remember to check that the back pressure does not increase too much.

For best performance and convenience use prepacked SEC columns

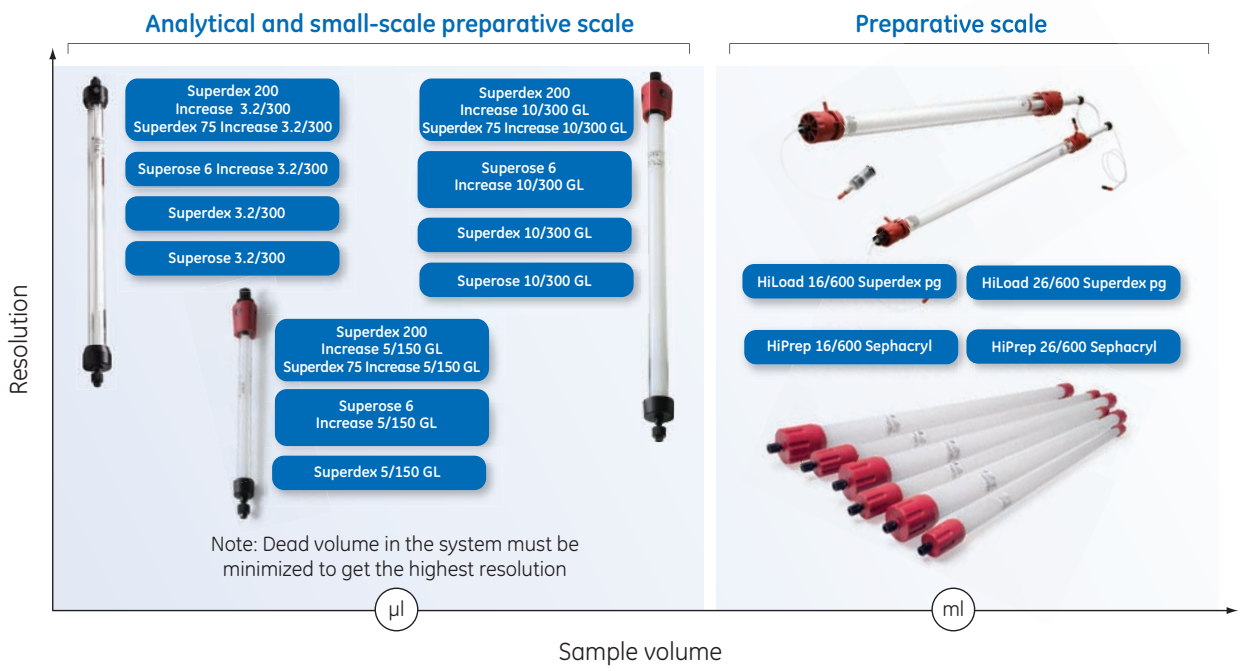


Fig 6. Schematic overview of resolution and sample volume for prepacked, high-resolution SEC columns.

Ordering information

Prepacked columns	VWR Cat. No
Superdex Peptide 3.2/300	**10048-752**
Superdex Peptide 10/300 GL	**97067-864**
Superdex 75 Increase 3.2/300	**75799-304**
Superdex 75 Increase 10/300 GL	**75799-300**
Superdex 75 Increase 5/150 GL	**75799-302**
Superdex 75 3.2/300*	**10048-734**
Superdex 75 10/300 GL*	**97067-860**
Superdex 75 5/150 GL*	**97067-868**
Superdex 200 Increase 3.2/300	**89497-276**
Superdex 200 Increase 10/300 GL	**89497-272**
Superdex 200 Increase 5/150 GL	**89497-274**
HiLoad 16/600 Superdex 30 pg	**71002-662**
HiLoad 26/600 Superdex 30 pg	**71002-664**
HiLoad 16/600 Superdex 75 pg	**71002-666**
HiLoad 26/600 Superdex 75 pg	**71002-668**
HiLoad 16/600 Superdex 200 pg	**71002-670**
HiLoad 26/600 Superdex 200 pg	**71002-672**
Superose 12 3.2/300	**10048-756**
Superose 12 10/300 GL	**97067-858**
Superose 6 Increase 3.2/300	**10192-226**
Superose 6 Increase 10/300 GL	**10192-228**
Superose 6 Increase 5/150 GL	**10192-230**
HiPrep 16/60 Sephacryl S-100 HR	**95056-000**
HiPrep 26/60 Sephacryl S-100 HR	**95055-806**
HiPrep 16/60 Sephacryl S-200 HR	**95056-002**
HiPrep 26/60 Sephacryl S-200 HR	**95055-808**
HiPrep 16/60 Sephacryl S-300 HR	**95056-004**
HiPrep 26/60 Sephacryl S-300 HR	**95055-810**
HiPrep 16/60 Sephacryl S-400 HR	**97067-768**
HiPrep 26/60 Sephacryl S-400 HR	**97067-770**
HiPrep 16/60 Sephacryl S-500 HR	**97067-772**
HiPrep 26/60 Sephacryl S-500 HR	**97067-774**
HiTrap Desalting (1 × 5 ml)	**89501-384**
HiTrap Desalting (5 × 5 ml)	**95056-006**
HiTrap Desalting (100 × 5 ml)	**97068-014**
HiPrep 26/10 Desalting (1 × 53 ml)	**95055-814**
HiPrep 26/10 Desalting (4 × 53 ml)	**95055-870**
PD-10 Desalting Columns (30 pcs)	**95017-001**

* Superdex 75 5/150 GL, 3.2/300 and 10/300 GL columns are to be replaced by new generation Superdex 75 Increase columns. Superdex 75 columns will be available until December 15, 2018.

Chromatography media	Pack size	VWR Cat. No.
Superdex 30 prep grade	150 ml	**95017-026**
Superdex 75 prep grade	150 ml	**95017-072**
Superdex 200 prep grade	150 ml	**95017-068**
Superose 12 prep grade	125 ml	**95055-854**
Superose 6 prep grade	125 ml	**95055-852**
Sephacryl S-100 HR	150 ml	**95016-916**
	750 ml	**95016-914**
Sephacryl S-200 HR	150 ml	**95016-904**
	750 ml	**95016-902**
Sephacryl S-300 HR	150 ml	**95016-908**
	750 ml	**95016-906**
Sephacryl S-400 HR	150 ml	**95016-912**
	750 ml	**95016-910**
Sephacryl S-500 HR	150 m	**95016-920**
	750 ml	**95016-918**
Sephacryl S-1000 SF	750 ml	**95016-856**
Sephadex G-10	100 g	**95016-746**
	500 g	**95016-748**
Sephadex G-25 Superfine	100 g	**95016-754**
Sephadex G-25 Fine	100 g	**95016-756**
	500 g	**95016-758**
Sephadex G-25 Medium	100 g	**95016-760**
	500 g	**95016-762**
Sephadex G-50 Fine	100 g	**95016-772**
	500 g	**95016-774**
Sephadex LH-20	25 g	**95016-800**
	100 g	**95016-796**
	500 g	**95016-798**

Related products	
Gel Filtration Calibration Kit, LMW	**95017-646**
Gel Filtration Calibration Kit, HMW	**95017-648**
Handbook: Size Exclusion Chromatography, Principles and Methods	**-**

Selection guide SEC media

Start here

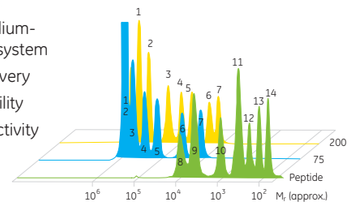
Group separation

High-resolution fractionation

Preparative or analytical; high resolution (M_r 100-600 000)

Superdex

High/medium-pressure system
High recovery
High stability
High selectivity



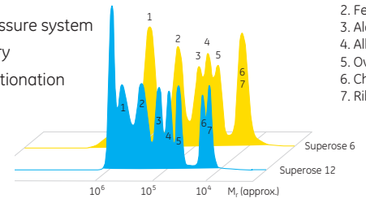
1. Thyroglobulin (M_r 669 000)
2. Ferritin (M_r 440 000)
3. Aldolase (M_r 158 000)
4. Albumin (M_r 67 000)
5. Ovalbumin (M_r 43 000)
6. Chymotrypsinogen A (M_r 25 000)
7. Ribonuclease A (M_r 13 700)
8. Cytochrome C (M_r 12 500)
9. Aprotinin (M_r 6500)
10. Gastrin I (M_r 2126)
11. Substance P (M_r 1348)
12. (Gly) 6 (M_r 260)
13. (Gly) 3 (M_r 189)
14. Gly (M_r 75)

- Micro preparative Analytical separation
  - Peptides → Superdex Peptide
  - Small proteins, Polynucleotides → Superdex 75 Increase*, Superdex 75
  - Proteins, DNA-fragments → Superdex 200 Increase*, Superdex 200
- Preparative separation
  - Peptides → Superdex 30 prep grade
  - Small proteins, Polynucleotides → Superdex 75 prep grade
  - Proteins, DNA fragments → Superdex 200 prep grade

Preparative or analytical; wide size range (M_r 1000-5 × 10^6)

Superose

Medium-pressure system
High recovery
Wide M_r fractionation range



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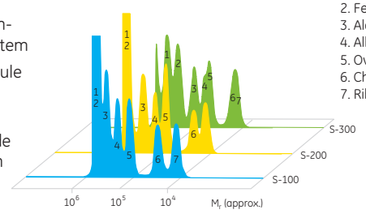
3. Aldolase (M_r 158 000)
4. Albumin (M_r 67 000)
5. Ovalbumin (M_r 43 000)
6. Chymotrypsinogen A (M_r 25 000)
7. Ribonuclease A (M_r 13 700)

- Micropreparative Analytical separation
  - Small proteins up to DNA fragments → Superose 6 Increase*, Superose 6
  - Small/medium proteins → Superose 12
- Preparative separation
  - Small proteins up to DNA fragments → Superose 6 prep grade
  - Small/medium proteins → Superose 12 prep grade

Preparative; larger biomolecules (M_r 1000–10^8)

Sephacryl

Low/medium-pressure system
Macromolecule separation
Product line covering wide fractionation range



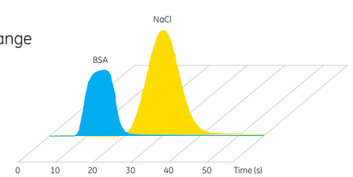
1. Thyroglobulin (M_r 669 000)
2. Ferritin (M_r 440 000)
3. Aldolase (M_r 158 000)
4. Albumin (M_r 67 000)
5. Ovalbumin (M_r 43 000)
6. Chymotrypsinogen A (M_r 25 000)
7. Ribonuclease A (M_r 13 700)

- Proteins
  - Small proteins → Sephacryl S-100 HR
  - Medium proteins → Sephacryl S-200 HR
  - Large proteins → Sephacryl S-300 HR
- Macromolecules
  - Small macromolecules → Sephacryl S-400 HR
  - Large macromolecules → Sephacryl S-500 HR
- Small particles, viruses → Sephacryl S-1000 SF

Desalting/Buffer exchange

Sephadex

Desalting
Buffer exchange

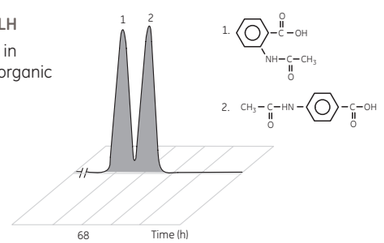


- Small peptides → Sephadex G-10
- Peptides/small proteins → Sephadex G-25 SF, Sephadex G-25 F, Sephadex G-25 M
- Proteins → Sephadex G-50 F

In organic solvents

Sephadex LH

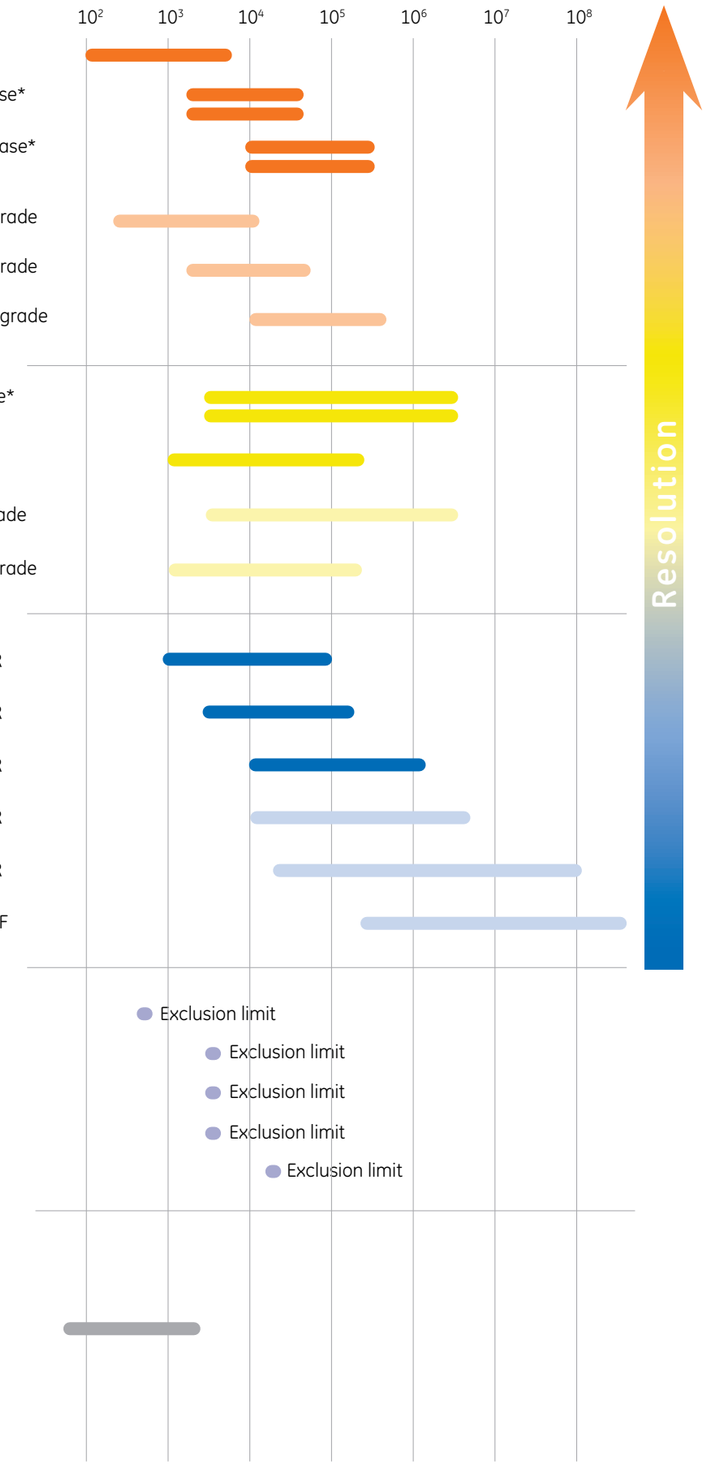
Separation in (nonpolar) organic solvent



- Low molecular weight steroids, Terpenoids, lipids, and peptides → Sephadex LH-20

* *The highest resolution is obtained with the new generation SEC media: Superdex Increase and Superose Increase.*

Fractionation range
(globular proteins)



Product		Ordering information			Fractionation range globular proteins M_r (relative molecular weight)	Fractionation range dextrans M_r (relative molecular weight)	Exclusion limit DNA (base pairs)	Average particle size (µm)	Column efficiency (N/m)	pH stability‡‡ (long term)	Maximum or typical pressure drop over the packed bed†† (MPa/psi)	Maximum operating flow rate††	Recommended operational flow rate	Recommended sample volume	Approx. bed volume (ml)	Applications
Prepacked column/Bulk media		VWR Cat. No.	Column dim. i.d. × bed height (mm)	Pack size												
Group separation																
HiTrap™ Desalting HiTrap Desalting‡	B	**95056-006** **97068-014**	16 × 25 16 × 25	5 100	1000–5000	100–5000	10	15–90	Not specified	2 to 13	0.3/44	15 ml/min		0.25 to 1.5 ml	5	Fast and convenient group separation between high and low molecular weight substances
HiPrep 26/10 Desalting HiPrep 26/10 Desalting	B	**95055-814** **95055-870**	26 × 100 26 × 100	1 4	1000–5000	100–5000	10	20–80 (dry)	Not specified	2 to 13	0.15/22	40 ml/min		2.5 to 15 ml	53	Fast and convenient group separation between high and low molecular weight substances
PD-10 Desalting Columns††	B	**95017-001**	14.7 × 50	1	1000–5000	100–5000	10	86–258	Not specified	2 to 13	–	–		1.5 to 2.5 ml	8.3	Disposable column for group separation and buffer exchange
Sephadex G-10†	B	**95016-746** **95016-748**	– –	100 g 500 g	> 700	> 700	2	40–120 (dry)	–	2 to 13	Can be calculated using Darcy's law	40 cm/h§		–	–	Fast and convenient group separation between peptides and low molecular weight substances
Sephadex G-25 Superfine†	B	**95016-754**	–	100 g	1000–5000	100–5000	10	20–50 (dry)	–	2 to 13		20 cm/h§		–	–	Fast and convenient group separation between high and low molecular weight substances
Sephadex G-25 Fine†	B	**95016-756** **95016-758**	– –	100 g 500 g	1000–5000	100–5000	10	20–80 (dry)	–	2 to 13		60 cm/h§		–	–	
Sephadex G-25 Medium†		**95016-760** **95016-762**	– –	100 g 500 g	1000–5000	100–5000	10	50–150 (dry)	–	2 to 13		150 cm/h§		–	–	
Sephadex G-50 Fine†		**95016-772** **95016-774**	– –	100 g 500 g	1000–30 000	500–10 000	No data	20–80 (dry)	–	2 to 10		60 cm/h§		–	–	
Sephadex LH-20†		**95016-800** **95016-796** **95016-798**	– – –	25 g 100 g 500 g	< 5000	No data	–	27–163 (dry)	–	2 to 11	0.15/22	30 cm/h§		–	–	Separation of natural products, such as steroids, terpenoids and lipids, in organic solvents
High-resolution fractionation																
Superdex Peptide 3.2/300 Superdex Peptide 10/300 GL		**10048-752** **97067-864**	3.2 × 300 10 × 300	1 1	100–7000 100–7000	No data No data	No data No data	13 13	> 30 000 > 30 000	1 to 14 1 to 14	2.0/290 1.8/260	0.15 ml/min 1.2 ml/min	0.05 ml/min < 1 ml/min	4 to 50 µl 25 to 500 µl	2.4 24	Small-scale preparative and analytical high-resolution separation of peptides and other small biomolecules
Superdex 75 3.2/300* Superdex 75 10/300 GL* Superdex 75 5/150 GL*		**10048-734** **97067-860** **97067-868**	3.2 × 300 10 × 300 5 × 150	1 1 1	3000–70 000 3000–70 000 3000–70 000	500–30 000 500–30 000 500–30 000	No data No data No data	13 13 13	> 30 000 > 30 000 > 25 000	3 to 12 3 to 12 3 to 12	2.4/350 1.8/260 1.8/260	0.1 ml/min 1.5 ml/min 0.7 ml/min	0.05 ml/min 0.75 ml/min 0.3 ml/min	4 to 50 µl 25 to 500 µl 4 to 50 µl	2.4 24 3	Small-scale preparative and analytical high-resolving separation of proteins, small proteins and polynucleotides, e.g., recombinant tagged proteins Rapid size analysis of protein homogeneity in screening experiments
Superdex 75 Increase 3.2/300 Superdex 75 Increase 10/300 GL Superdex 75 Increase 5/150 GL		**75799-304** **75799-300** **75799-302**	3.2 × 300 10 × 300 5 × 150	1 1 1	3000–70 000 3000–70 000 3000–70 000	500–30 000 500–30 000 500–30 000	No data No data No data	9 9 9	> 43 000 > 43 000 > 38 000	3 to 12 3 to 12 3 to 12	2.0/290 3.0/435 3.0/435	0.15 ml/min 1.6 ml/min 0.75 ml/min	0.075 ml/min 0.8 ml/min 0.45 ml/min	4 to 50 µl 25 to 500 µl 4 to 50 µl	2.4 24 3	Small-scale preparative purification and analysis of proteins, such as recombinant tagged proteins, when small sample and buffer consumption is important Standard for small-scale preparative purification and analysis of proteins, such as recombinant tagged proteins Rapid purity check and homogeneity analysis of proteins, such as recombinant tagged proteins.
Superdex 200 Increase 3.2/300 Superdex 200 Increase 10/300 GL Superdex 200 Increase 5/150 GL		**89497-276** **89497-272** **89497-274**	3.2 × 300 10 × 300 5 × 150	1 1 1	10 000–600 000 10 000–600 000 10 000–600 000	1000–100 000 1000–100 000 1000–100 000		8.6 8.6 8.6	> 48 000 > 48 000 > 42 000	3 to 12 3 to 12 3 to 12	3.0/435 3.0/435 3.0/435	0.15 ml/min 1.8 ml/min 0.75 ml/min	0.075 ml/min 0.75 ml/min 0.45 ml/min	4 to 50 µl 25 to 500 µl 4 to 50 µl	2.4 24 3	Small-scale preparative purification and analysis of proteins, especially monoclonal antibodies when small sample and buffer consumption is important Standard for small-scale preparative purification and analysis of proteins, especially monoclonal antibodies. Rapid purity check and homogeneity analysis of proteins, especially monoclonal antibodies
HiLoad 16/600 Superdex 30 pg HiLoad 26/600 Superdex 30 pg	B	**71002-662** **71002-664**	16 × 600 26 × 600	1 1	< 10 000 < 10 000	No data No data	No data No data	34 34	> 13 000 > 13 000	3 to 12 3 to 12	0.3/42 0.3/42	1.7 ml/min 4.4 ml/min	1.0 ml/min 2.6 ml/min	≤ 5 ml ≤ 13 ml	120 320	Preparative separation of peptides and other small biomolecules
HiLoad 16/600 Superdex 75 pg HiLoad 26/600 Superdex 75 pg	B	**71002-666** **71002-668**	16 × 600 26 × 600	1 1	3000–70 000 3000–70 000	500–30 000 500–30 000	No data No data	34 34	> 13 000 > 13 000	3 to 12 3 to 12	0.3/42 0.3/42	1.7 ml/min 4.4 ml/min	1.0 ml/min 2.6 ml/min	≤ 5 ml ≤ 13 ml	120 320	Rapid, preparative separation of proteins, peptides, polynucleotides, and other biomolecules
HiLoad 16/600 Superdex 200 pg HiLoad 26/600 Superdex 200 pg	B	**71002-670** **71002-672**	16 × 600 26 × 600	1 1	10 000–600 000 10 000–600 000	1000–100 000 1000–100 000	No data No data	34 34	> 13 000 > 13 000	3 to 12 3 to 12	0.3/42 0.3/42	1.7 ml/min 4.4 ml/min	1.0 ml/min 2.6 ml/min	≤ 5 ml ≤ 13 ml	120 320	Rapid, preparative separation of proteins, especially monoclonal antibodies, DNA fragments, and other biomolecules
Superdex 30 prep grade†	B	**95017-026**		150 ml	< 10 000	No data	No data	34	–	3 to 12	0.3/42	90 cm/h§	10-50 cm/h	–	–	Preparative separation of peptides and other small biomolecules
Superdex 75 prep grade†	B	**95017-072**		150 ml	3000–70 000	500–30 000	No data	34	–	3 to 12	0.3/42	90 cm/h§	10-50 cm/h	–	–	Rapid, preparative separation of proteins, peptides, polynucleotides, and other biomolecules
Superdex 200 prep grade†	B	**95017-068**		150 ml	10 000–600 000	1000–100 000	No data	34	–	3 to 12	0.3/42	90 cm/h§	10-50 cm/h	–	–	Rapid, preparative separation of proteins, especially monoclonal antibodies, DNA fragments, and other biomolecules
Superose 12 3.2/300 Superose 12 10/300 GL		**10048-756** **97067-858**	3.2 × 300 10 × 300	1 1	1000–300 000 1000–300 000	No data No data	150 150	11 11	> 40 000 > 40 000	3 to 12 3 to 12	2.4/350 3/435	0.1 ml/min 1.5 ml/min	0.04 ml/min 0.5 ml/min	4 to 50 µl 25 to 500 µl	2.4 24	Small-scale preparative and analytical high-resolving separation of proteins, peptides, oligonucleotides, and polysaccharides
Superose 6 Increase 3.2/300 Superose 6 Increase 10/300 GL Superose 6 Increase 5/150 GL		**10192-226** **10192-228** **10192-230**	3.2 × 300 10 × 300 5 × 150	1 1 1	5 000–5 000 000 5 000–5 000 000 5 000–5 000 000	1 000–300 000 1 000–300 000 1 000–300 000		8.6 8.6 8.6	> 48 000 > 48 000 > 42 000	3 to 12 3 to 12 3 to 12	3.0/435 3.0/435 3.0/435	0.15 ml/min 1.5 ml/min 0.75 ml/min	0.04 ml/min 0.5 ml/min 0.3 ml/min	4 to 50 µl 25 to 500 µl 4 to 50 µl	2.4 24 3	Small-scale preparative purification and anlysis of large proteins and other biomolecules, when small sample and buffer consumption is important Standard for small-scale preparative purification and analysis of large proteins and other biomolecules, especially protein complexes Rapid purity check and homogeneity analysis of large proteins and protein complexes
Superose 12 prep grade		**95055-854**	–	125 ml	1000–300 000	No data	150	30	–	3 to 12	0.7/100	40 cm/h§	up to 40 cm/h		–	Preparative high-performance separation of proteins, peptides, oligonucleotides, and polysaccharides
Superose 6 prep grade		**95055-852**	–	125 ml	5000–5 000 000	No data	450	30	–	3 to 12	0.4/58	40 cm/h§	up to 40 cm/h		–	Preparative high-performance separation of proteins, peptides, oligonucleotides, polysaccharides, and nucleic acids
HiPrep 16/60 Sephacryl S-100 HR HiPrep 26/60 Sephacryl S-100 HR	B	**95056-000** **95055-806**	16 × 600 26 × 600	1 1	1000–100 000 1000–100 000	No data No data	No data No data	47 47	> 5000 > 5000	3 to 11 3 to 11	0.15/22 0.15/22	1.0 ml/min 2.7 ml/min	0.5 ml/min 1.3 ml/min	≤ 5 ml ≤ 13 ml	120 320	Preparative separation of proteins and peptides
HiPrep 16/60 Sephacryl S-200 HR HiPrep 26/60 Sephacryl S-200 HR	B	**95056-002** **95055-808**	16 × 600 26 × 600	1 1	5000–250 000 5000–250 000	1000–80 000 1000–80 000	30 30	47 47	> 5000 > 5000	3 to 11 3 to 11	0.15/22 0.15/22	1.0 ml/min 2.7 ml/min	0.5 ml/min 1.3 ml/min	≤ 5 ml ≤ 13 ml	120 320	Preparative separation of proteins e.g., small serum proteins such as albumin
HiPrep 16/60 Sephacryl S-300 HR HiPrep 26/60 Sephacryl S-300 HR	B	**95056-004** **95055-810**	16 × 600 26 × 600	1 1	10 000–1 500 000 10 000–1 500 000	2000–400 000 2000–400 000	118 118	47 47	> 5000 > 5000	3 to 11 3 to 11	0.15/22 0.15/22	1.0 ml/min 2.7 ml/min	0.5 ml/min 1.3 ml/min	≤ 5 ml ≤ 13 ml	120 320	Preparative separation of proteins e.g., membrane proteins and antibodies
Sephacryl S-100 HR†	B	**95016-916** **95016-914**	– –	150 ml 750 ml	1000–100 000	No data	No data	47	–	3 to 11	0.2/29	60 cm/h§	10–35 cm/h	–	–	Preparative separation of proteins and peptides
Sephacryl S-200 HR†	B	**95016-904** **95016-902**	– –	150 ml 750 ml	5000–250 000	1000–80 000	30	47	–	3 to 11	0.2/29	60 cm/h§	10–35 cm/h	–	–	Preparative separation of proteins e.g., small serum proteins such as albumin
Sephacryl S-300 HR†	B	**95016-908** **95016-906**	– –	150 ml 750 ml	10 000–1 500 000	2000–400 000	118	47	–	3 to 11	0.2/29	60 cm/h§	10–35 cm/h	–	–	Preparative separation of proteins e.g., membrane proteins and antibodies
HiPrep 16/60 Sephacryl S-400 HR HiPrep 26/60 Sephacryl S-400 HR	B	**97067-768** **97067-770**	16 × 600 26 × 600	1 1	20 000–8 000 000 20 000–8 000 000	10 000–2 000 000 10 000–2 000 000	271 271	47 47	> 5000 > 5000	3 to 11 3 to 11	0.15/22 0.15/22	1.0 ml/min 2.7 ml/min	0.5 ml/min 1.3 ml/min	≤ 5 ml ≤ 13 ml	120 320	Preparative separation of polysaccharides and other macromolecules with extended structures e.g., proteoglycans and liposomes
HiPrep 16/60 Sephacryl S-500 HR HiPrep 26/60 Sephacryl S-500 HR	B	**97067-772** **97067-774**	16 × 600 26 × 600	1 1	No data No data	40 000–20 000 000 40 000–20 000 000	1078 1078	47 47	> 5000 > 5000	3 to 11 3 to 11	0.15/22 0.15/22	1.0 ml/min 2.7 ml/min	0.5 ml/min 1.3 ml/min	≤ 5 ml ≤ 13 ml	120 320	Preparative separation of large macromolecules e.g., group separation of DNA restriction fragments
Sephacryl S-400 HR†	B	**95016-912** **95016-910**	– –	150 ml 750 ml	20 000–8 000 000	10 000–2 000 000	271	47	–	3 to 11	0.2/29	60 cm/h§	10–35 cm/h	–	–	Preparative separation of polysaccharides and other macromolecules with extended structures e.g., proteoglycans and liposomes
Sephacryl S-500 HR†	B	**95016-920** **95016-918**	– –	150 ml 750 ml	No data	40 000–20 000 000	1078	47	–	3 to 11	0.2/29	50 cm/h§	10–35 cm/h	–	–	Preparative separation of large macromolecules e.g., group separation of DNA restriction fragments
Sephacryl S-1000 SF†		**95016-856**	–	750 ml	–	500 000–100 000 000	20 000	65	–	3 to 11	Not determined	40 cm/h§	2–30 cm/h	–	–	Preparation of DNA and separation of very large polysaccharides, proteoglycans, and small particles e.g., membrane-bound vesicles and viruses

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* Superdex 75 3.2/300, 5/150 GL and 10/300 GL columns are being replaced by new generation Superdex 75 Increase columns. Superdex 75 columns will be available until December 15, 2018.

§ Flow rate is calculated from measurement in packed columns with an i.d. of 2.6 cm. A column heigth of 60 cm is used for Superose, Superdex, and Sephacryl. For Sephadex, the column i.d. is 2.6 cm and the height 30 cm.

†† Labmate buffer reservoir (18321603) can be used with PD-10 Desalting Columns for easier and more convenient equilibration.

‡‡ pH stability long term refers to the pH interval where the chromatography medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance. All ranges given are estimates based on our knowledge and experience.

†† At room temperature in aqueous buffer. The flow rate giving optimal resolution depends on the sample. Refer to instructions for each column and media. Use lower flow rate for viscous solutions and low temperature.

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