Selection guide

Size exclusion chromatography columns and resins

cytiva

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 resin packed in a column. SEC resins consist of spherical chromatography particles (chromatography beads) 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 resins 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.

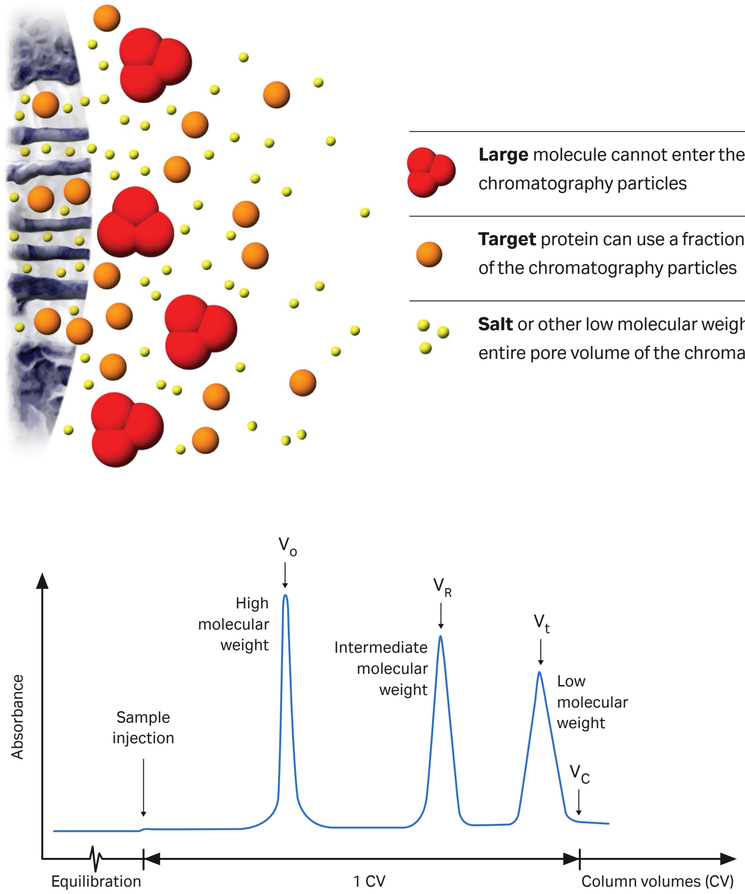


Fig 1. Schematic process of SEC.

Size exclusion chromatography 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.

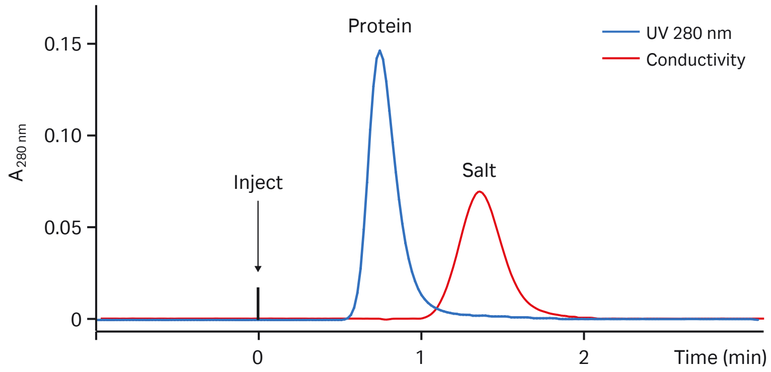


Fig 2. Typical group separation.

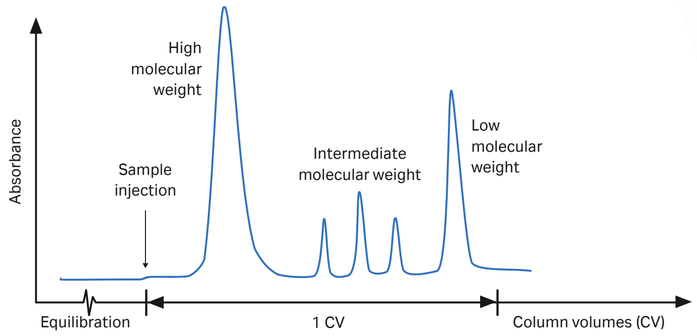


Fig 3. Typical high-resolution SEC separation.

Chromatography resin 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 resin 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, suitable for laboratory scale.

Note: The highest resolution is obtained with the new generation SEC resins: 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 size exclusion chromatography resins

Resolution is a function of the selectivity of the SEC resin, that is the distance between two peaks, and the efficiency of the resin, 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 resin 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 resin 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 resin. The smaller the bead size, the higher the resolution.

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

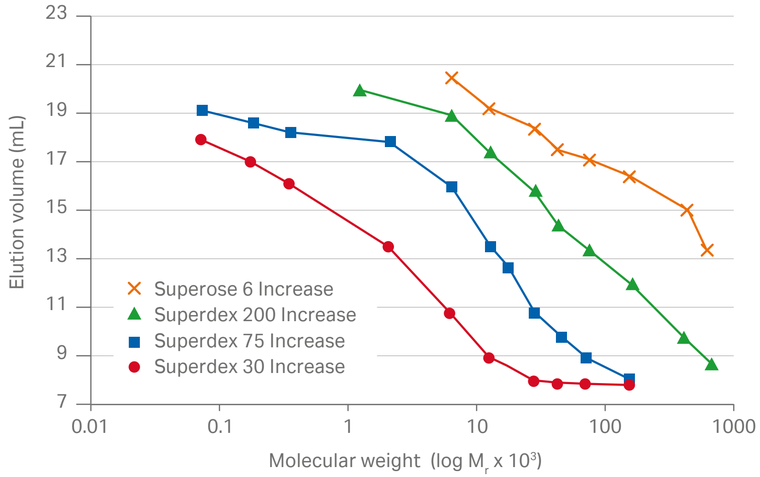


Fig 4. Selectivity curves for Superdex 30 Increase, Superdex 75 Increase, Superdex 200 Increase and Superose 6 Increase. Note that the whole fractionation range of Superose 6 Increase is not covered in this diagram.

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 SEC resin, 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 resin 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 resin.

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.

Cytiva's 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 cytiva.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 resin 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 resin, 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

Cytiva 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.

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 resins 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.

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)

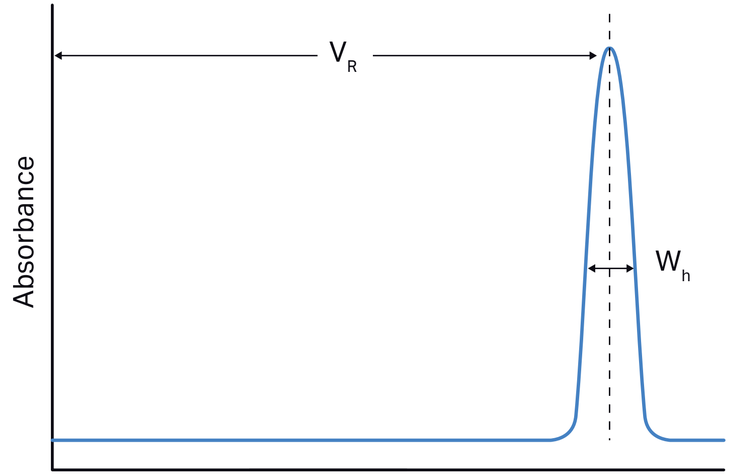


Fig 5. Column efficiency test.

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 Cytiva or downloaded at cytiva.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 resin	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 chromatography 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

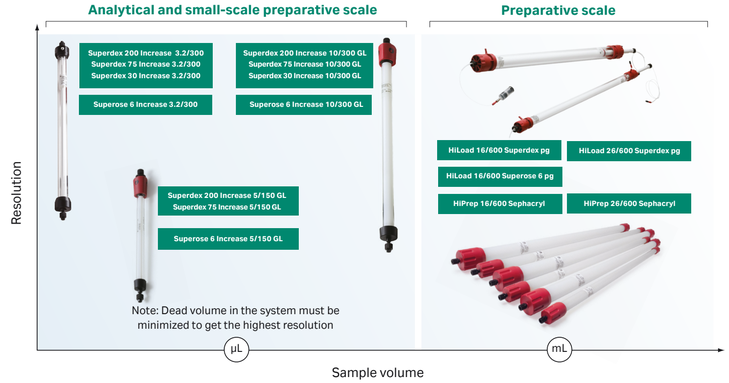


Fig 6. Schematic overview of resolution and sample volume for prepacked, high-resolution SEC columns.

Ordering information

Prepacked columns	Product code
Superdex 30 Increase 3.2/300*	29219758
Superdex 30 Increase 10/300 GL*	29219757
Superdex 75 Increase 3.2/300	29148723
Superdex 75 Increase 10/300 GL	29148721
Superdex 75 Increase 5/150 GL	29148722
Superdex 200 Increase 3.2/300	28990946
Superdex 200 Increase 10/300 GL	28990944
Superdex 200 Increase 5/150 GL	28990945
HiLoad 16/600 Superdex 30 pg	28989331
HiLoad 26/600 Superdex 30 pg	28989332
HiLoad 16/600 Superdex 75 pg	28989333
HiLoad 26/600 Superdex 75 pg	28989334
HiLoad 16/600 Superdex 200 pg	28989335
HiLoad 26/600 Superdex 200 pg	28989336
HiLoad 16/600 Superose 6 pg	29323952
Superose 6 Increase 3.2/300	29091598
Superose 6 Increase 10/300 GL	29091596
Superose 6 Increase 5/150 GL	29091597

* Superdex 30 Increase columns are replacing Superdex Peptide columns.

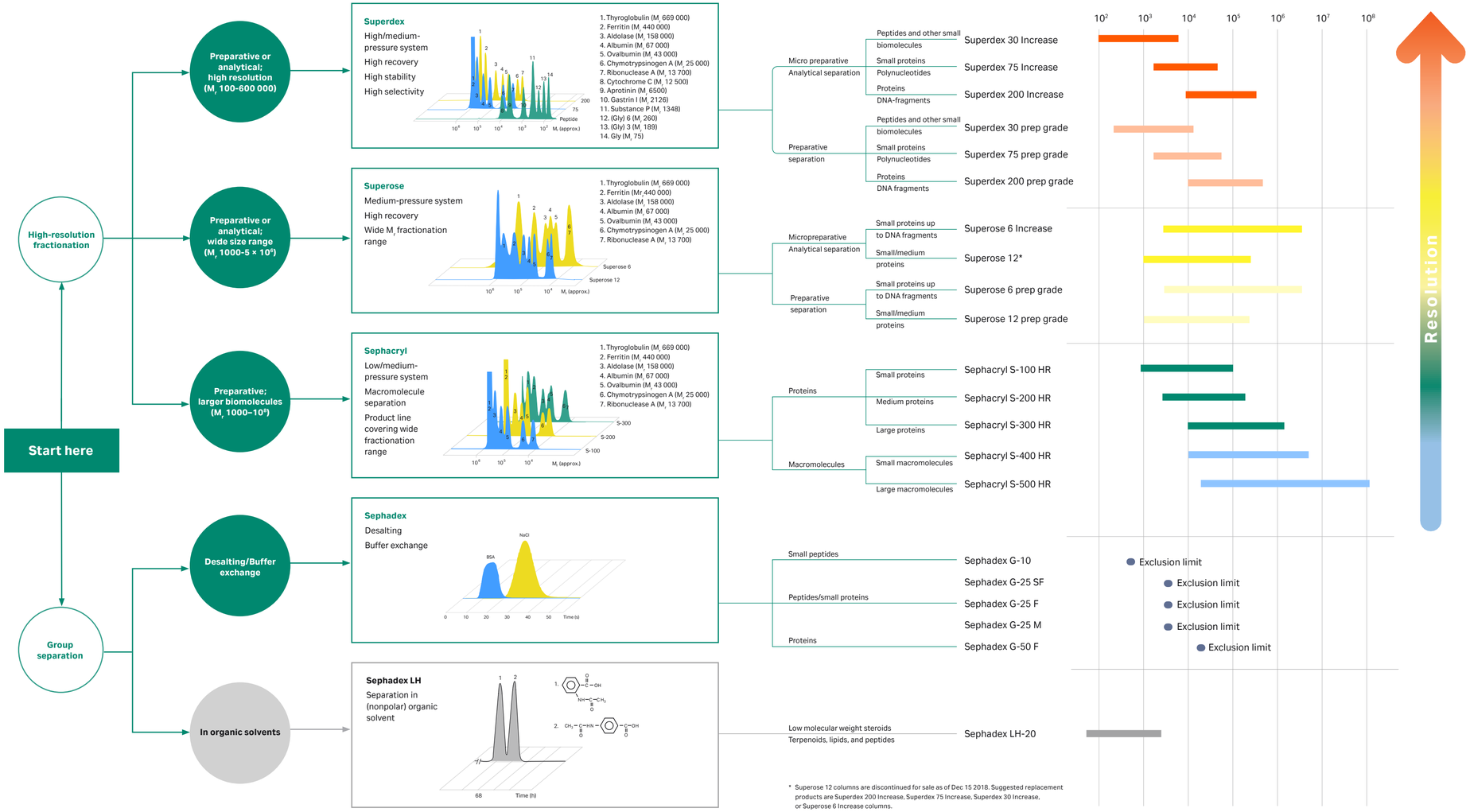
Prepacked columns	Product code
HiPrep 16/60 Sephacryl S-100 HR	17116501
HiPrep 26/60 Sephacryl S-100 HR	17119401
HiPrep 16/60 Sephacryl S-200 HR	17116601
HiPrep 26/60 Sephacryl S-200 HR	17119501
HiPrep 16/60 Sephacryl S-300 HR	17116701
HiPrep 26/60 Sephacryl S-300 HR	17119601
HiPrep 16/60 Sephacryl S-400 HR	28935604
HiPrep 26/60 Sephacryl S-400 HR	28935605
HiPrep 16/60 Sephacryl S-500 HR	28935606
HiPrep 26/60 Sephacryl S-500 HR	28935607
HiTrap Desalting (1 × 5 mL)	29048684
HiTrap Desalting (5 × 5 mL)	17140801
HiPrep 26/10 Desalting (1 × 53 mL)	17508701
HiPrep 26/10 Desalting (4 × 53 mL)	17508702
PD-10 Desalting Columns (30 pcs)	17085101

Chromatography resins	Pack size	Product code
Superdex 30 prep grade	150 mL	17090501
Superdex 75 prep grade	150 mL	17104401
Superdex 200 prep grade	150 mL	17104301
Superose 12 prep grade	125 mL	17053601
Superose 6 prep grade	125 mL	17048901
Sephacryl S-100 HR	150 mL	17061210
	750 mL	17061201
Sephacryl S-200 HR	150 mL	17058410
	750 mL	17058401
Sephacryl S-300 HR	150 mL	17059910
	750 mL	17059901
Sephacryl S-400 HR	150 mL	17060910
	750 mL	17060901
Sephacryl S-500 HR	150 mL	17061310
	750 mL	17061301
Sephacryl S-1000 SF	750 mL	17047601
Sephadex G-10	100 g	17001001
	500 g	17001002
Sephadex G-25 Superfine	100 g	17003101
Sephadex G-25 Fine	100 g	17003201
	500 g	17003202
Sephadex G-25 Medium	100 g	17003301
	500 g	17003302
Sephadex G-50 Fine	100 g	17004201
	500 g	17004202
Sephadex LH-20	25 g	17009010
	100 g	17009001
	500 g	17009002

Related products	Product code
Gel Filtration Calibration Kit, LMW	28403841
Gel Filtration Calibration Kit, HMW	28403842
Handbook: *Size Exclusion Chromatography, Principles and Methods*	18102218

Selection guide

Size exclusion chromatography resins



* Superose 12 columns are discontinued for sale as of Dec 15 2018. Suggested replacement products are Superdex 200 Increase, Superdex 75 Increase, Superdex 30 Increase, or Superose 6 Increase columns.

Fractionation range

(globular proteins)

Product		Ordering information			Fractionation range (approx.) globular proteins M_r (relative molecular weight)	Fractionation range (approx.) dextrans M_p (peak molecular weight)	Approximate exclusion limit DNA (base pairs)	Particle size range (µm)	Column efficiency (N/m)	pH stability[‡‡] (operational and cleaning-in-place)	Maximum or typical pressure drop over the packed bed[¶¶] (MPa/psi)	Recommended maximum operating flow	Recommended operating flow	Recommended sample volume	Approx. bed volume (mL)	Applications
Prepacked column/Bulk resins		**Product code**	**Column dim. i.d. × bed height (mm)**	**Pack size**												
Group separation																
HiTrap™ Desalting	B	17140801	16 × 25	5	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
HiTrap Desalting‡		29048684	16 × 25	1												
HiPrep 26/10 Desalting	B	17508701	26 × 100	1	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
HiPrep 26/10 Desalting		17508702	26 × 100	4												
PD-10 Desalting Columns††	B	17085101	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	17001001	–	100 g	> 700	> 700	2	40–120 (dry)	–	2 to 13	–	40 cm/h§		–	–	Fast and convenient group separation between peptides and low molecular weight substances
		17001002	–	500 g												
Sephadex G-25 Superfine†	B	17003101	–	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	17003201	–	100 g	1000–5000	100–5000	10	20–80 (dry)	–	2 to 13	–	60 cm/h§		–	–	
		17003202	–	500 g												
Sephadex G-25 Medium†		17003301	–	100 g	1000–5000	100–5000	10	50–150 (dry)	–	2 to 13	–	150 cm/h§		–	–	
		17003302	–	500 g												
Sephadex G-50 Fine†		17004201	–	100 g	1000–30 000	500–10 000	No data	20–80 (dry)	–	2 to 10	–	60 cm/h§		–	–	
		17004202	–	500 g												
Sephadex LH-20†		17009010	–	25 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
		17009001	–	100 g												
		17009002	–	500 g												
High-resolution fractionation																
Superdex 30 Increase 3.2/300*		29219758	3.2 × 300	1	100–7000	No data	No data	9	> 38 000	3 to 12	2.0/290	0.15 mL/min	0.075 mL/min	4 to 50 µL	2.4	High sensitivity for small sample volumes of peptides and other small biomolecules
Superdex 30 Increase 10/300 GL*		29219757	10 × 300	1	100–7000	No data	No data	9	> 43 000	3 to 12	3.0/435	1.2 mL/min	0.8 mL/min	25 to 500 µl	24	Standard for small-scale preparative purification and analysis of peptides and other small biomolecules
Superdex 75 Increase 3.2/300		29148723	3.2 × 300	1	3000–70 000	500–30 000	No data	9	> 43 000	3 to 12	2.0/290	0.15 mL/min	0.075 mL/min	4 to 50 µL	2.4	High sensitivity for small sample volumes of recombinant tagged proteins
Superdex 75 Increase 10/300 GL		29148721	10 × 300	1	3000–70 000	500–30 000	No data	9	> 43 000	3 to 12	3.0/435	1.6 mL/min	0.8 mL/min	25 to 500 µl	24	Standard for small-scale preparative purification and analysis of proteins, such as recombinant tagged proteins
Superdex 75 Increase 5/150 GL		29148722	5 × 150	1	3000–70 000	500–30 000	No data	9	> 38 000	3 to 12	3.0/435	0.75 mL/min	0.45 mL/min	4 to 50 µl	3	Rapid purity check and homogeneity analysis of proteins, such as recombinant tagged proteins
Superdex 200 Increase 3.2/300		28990946	3.2 × 300	1	10 000–600 000	1000–100 000		8.6	> 48 000	3 to 12	2.0/290	0.15 mL/min	0.075 mL/min	4 to 50 µL	2.4	High sensitivity for small sample volumes of antibodies
Superdex 200 Increase 10/300 GL		28990944	10 × 300	1	10 000–600 000	1000–100 000		8.6	> 48 000	3 to 12	3.0/435	1.8 mL/min	0.75 mL/min	25 to 500 µL	24	Standard for small-scale preparative purification and analysis of proteins, especially monoclonal antibodies
Superdex 200 Increase 5/150 GL		28990945	5 × 150	1	10 000–600 000	1000–100 000		8.6	> 42 000	3 to 12	3.0/435	0.75 mL/min	0.45 mL/min	4 to 50 µL	3	Rapid purity check and homogeneity analysis of proteins, especially monoclonal antibodies
HiLoad 16/600 Superdex 30 pg	B	28989331	16 × 600	1	< 10 000	No data	No data	34	> 13 000	3 to 12	0.3/42	1.7 mL/min	1.0 mL/min	≤ 5 mL	120	Preparative separation of peptides and other small biomolecules
HiLoad 26/600 Superdex 30 pg		28989332	26 × 600	1	< 10 000	No data	No data	34	> 13 000	3 to 12	0.3/42	4.4 mL/min	2.6 mL/min	≤ 13 mL	320	
HiLoad 16/600 Superdex 75 pg	B	28989333	16 × 600	1	3000–70 000	500–30 000	No data	34	> 13 000	3 to 12	0.3/42	1.7 mL/min	1.0 mL/min	≤ 5 mL	120	Rapid, preparative separation of proteins, peptides, polynucleotides, and other biomolecules
HiLoad 26/600 Superdex 75 pg		28989334	26 × 600	1	3000–70 000	500–30 000	No data	34	> 13 000	3 to 12	0.3/42	4.4 mL/min	2.6 mL/min	≤ 13 mL	320	
HiLoad 16/600 Superdex 200 pg	B	28989335	16 × 600	1	10 000–600 000	1000–100 000	No data	34	> 13 000	3 to 12	0.3/42	1.7 mL/min	1.0 mL/min	≤ 5 mL	120	Rapid, preparative separation of proteins, especially monoclonal antibodies, DNA fragments, and other biomolecules
HiLoad 26/600 Superdex 200 pg		28989336	26 × 600	1	10 000–600 000	1000–100 000	No data	34	> 13 000	3 to 12	0.3/42	4.4 mL/min	2.6 mL/min	≤ 13 mL	320	
Superdex 30 prep grade†	B	17090501		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	17104401		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	17104301		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 6 Increase 3.2/300		29091598	3.2 × 300	1	5 000–5 000 000	1 000–300 000		8.6	> 48 000	3 to 12	2.0/290	0.15 mL/min	0.04 mL/min	4 to 50 µL	2.4	Small-scale preparative purification and anlysis of large proteins and other biomolecules, when small sample and buffer consumption is important
Superose 6 Increase 10/300 GL		29091596	10 × 300	1	5 000–5 000 000	1 000–300 000		8.6	> 48 000	3 to 12	3.0/435	1.5 mL/min	0.5 mL/min	25 to 500 µL	24	Standard for small-scale preparative purification and analysis of large proteins and other biomolecules, especially protein complexes
Superose 6 Increase 5/150 GL		29091597	5 × 150	1	5 000–5 000 000	1 000–300 000		8.6	> 42 000	3 to 12	3.0/435	0.75 mL/min	0.3 mL/min	4 to 50 µL	3	Rapid purity check and homogeneity analysis of large proteins and protein complexes
Superose 12 prep grade		17053601	–	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
HiLoad 16/600 Superose 6 pg		29323952	16 × 600	1	5000–5 000 000	No data	450	30	> 10 000	3 to 12	0.3/42	1.6 mL/min	1.0 mL/min	≤ 5 mL	120	Preparative separation of large proteins and other biomolecules, especially protein complexes
Superose 6 prep grade		17048901	–	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 large proteins and other biomolecules
HiPrep 16/60 Sephacryl S-100 HR	B	17116501	16 × 600	1	1000–100 000	No data	No data	47	> 5000	3 to 11	0.15/22	1.0 mL/min	0.5 mL/min	≤ 5 mL	120	Preparative separation of proteins and peptides
HiPrep 26/60 Sephacryl S-100 HR		17119401	26 × 600	1	1000–100 000	No data	No data	47	> 5000	3 to 11	0.15/22	2.7 mL/min	1.3 mL/min	≤ 13 mL	320	
HiPrep 16/60 Sephacryl S-200 HR	B	17116601	16 × 600	1	5000–250 000	1000–80 000	30	47	> 5000	3 to 11	0.15/22	1.0 mL/min	0.5 mL/min	≤ 5 mL	120	Preparative separation of proteins e.g., small serum proteins such as albumin
HiPrep 26/60 Sephacryl S-200 HR		17119501	26 × 600	1	5000–250 000	1000–80 000	30	47	> 5000	3 to 11	0.15/22	2.7 mL/min	1.3 mL/min	≤ 13 mL	320	
HiPrep 16/60 Sephacryl S-300 HR	B	17116701	16 × 600	1	10 000–1 500 000	2000–400 000	118	47	> 5000	3 to 11	0.15/22	1.0 mL/min	0.5 mL/min	≤ 5 mL	120	Preparative separation of proteins e.g., membrane proteins and antibodies
HiPrep 26/60 Sephacryl S-300 HR		17119601	26 × 600	1	10 000–1 500 000	2000–400 000	118	47	> 5000	3 to 11	0.15/22	2.7 mL/min	1.3 mL/min	≤ 13 mL	320	
Sephacryl S-100 HR†	B	17061210	– –	150 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
		17061201		750 mL												
Sephacryl S-200 HR†	B	17058410	– –	150 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
		17058401		750 mL												
Sephacryl S-300 HR†	B	17059910	– –	150 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
		17059901		750 mL												
HiPrep 16/60 Sephacryl S-400 HR	B	28935604	16 × 600	1	20 000–8 000 000	10 000–2 000 000	271	47	> 5000	3 to 11	0.15/22	1.0 mL/min	0.5 mL/min	≤ 5 mL	120	Preparative separation of polysaccharides and other macromolecules with extended structures e.g., proteoglycans and liposomes
HiPrep 26/60 Sephacryl S-400 HR		28935605	26 × 600	1	20 000–8 000 000	10 000–2 000 000	271	47	> 5000	3 to 11	0.15/22	2.7 mL/min	1.3 mL/min	≤ 13 mL	320	
HiPrep 16/60 Sephacryl S-500 HR	B	28935606	16 × 600	1	No data	40 000–20 000 000	1078	47	> 5000	3 to 11	0.15/22	1.0 mL/min	0.5 mL/min	≤ 5 mL	120	Preparative separation of large macromolecules e.g., group separation of DNA restriction fragments
HiPrep 26/60 Sephacryl S-500 HR		28935607	26 × 600	1	No data	40 000–20 000 000	1078	47	> 5000	3 to 11	0.15/22	2.7 mL/min	1.3 mL/min	≤ 13 mL	320	
Sephacryl S-400 HR†	B	17060910	– –	150 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
		17060901		750 mL												
Sephacryl S-500 HR†	B	17061310	– –	150 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
		17061301		750 mL												

B BioProcess™ resin—made for bioprocessing.

† Process-scale quantities are available. Please contact Cytiva for further information.

‡ Pack size available by special order.

* Superdex 30 Increase columns are replacing Superdex Peptide columns.

§ 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 range where resin can be operated without significant change in function.

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

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