Size exclusion chromatography columns and media

Selection guide

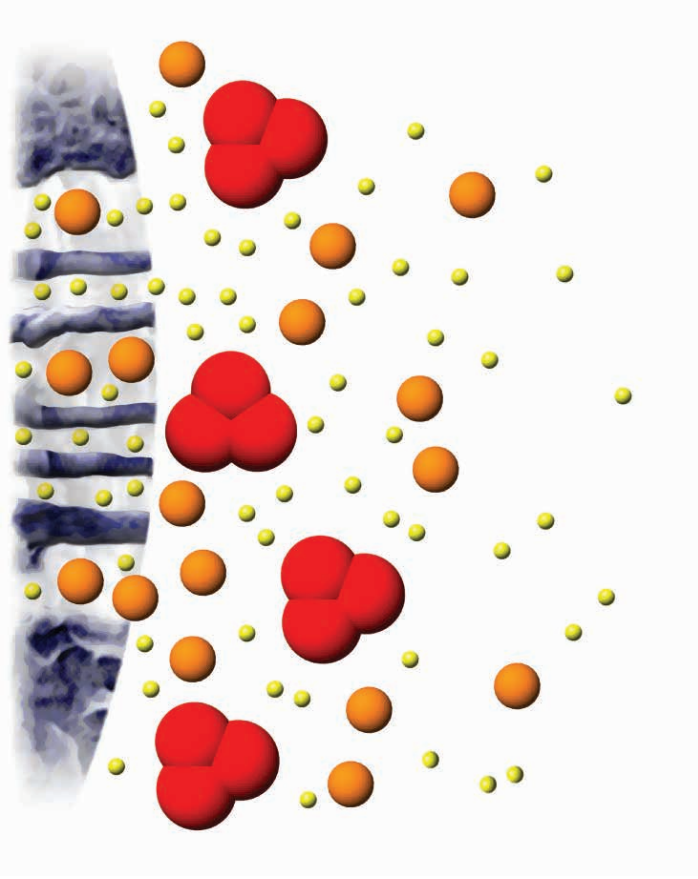
www.gelifesciences.com

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 consists 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 media with different selectivities are available and cover a molecular weight range from M_r 100 to 100 000 000, from peptides to very large proteins, protein complexes, and virus.



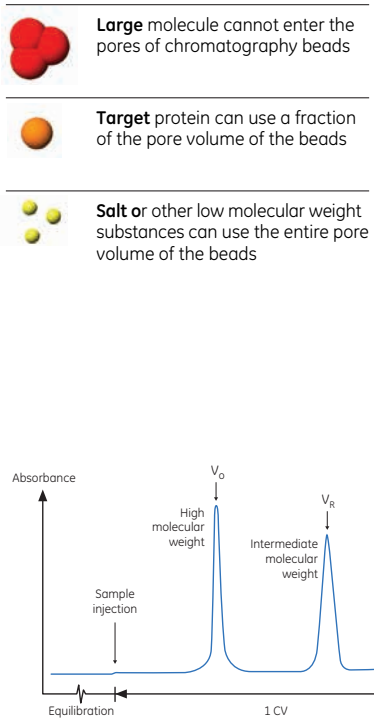


Fig 1. Schematic process of SEC.

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 gel filtration 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.

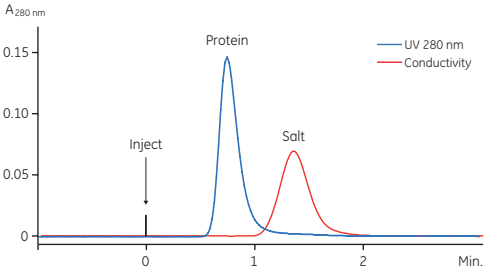


Fig 2. Typical group separation.

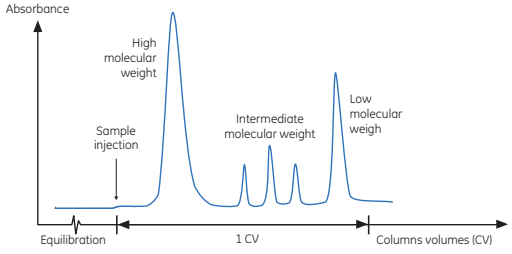


Fig 3. Typical high-resolution SEC separation.

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 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 next generation SEC media: Superdex Increase and Superose Increase.

Rapid purity check and screening

Superdex 75 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 medium

Resolution is a function of the selectivity of the 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 a GF 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 medium. The smaller the bead size, the higher the resolution.

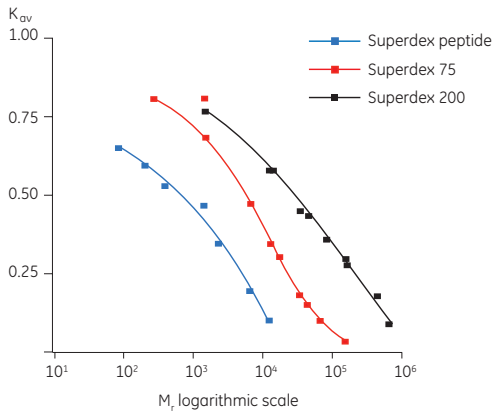


Fig 4. Selectivity curves for Superdex.

In cases where two media have a similar fractionation range, select the 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 a 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 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 time of the 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.

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 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 (2-8 degrees).

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 gel filtration 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 29-0272-71). 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)

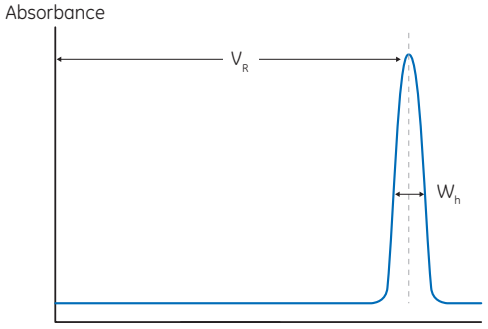


Fig 5. Column efficiency test.

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 may be decreased
Decrease sample volume	Improved resolution

Maintenance

Note: The description of regular cleaning below refers to Superdex and Superose columns; for other 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 base line 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 29-1407-60 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, cm/hour. To convert between linear flow and volumetric flow rate use the following formulas:

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

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

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

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

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 medium	Stop the flow. Close the outlet tubing with the domed nut and then disconnect the inlet tubing. Adjust the adaptor to the 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 pre packed size exclusion chromatography columns

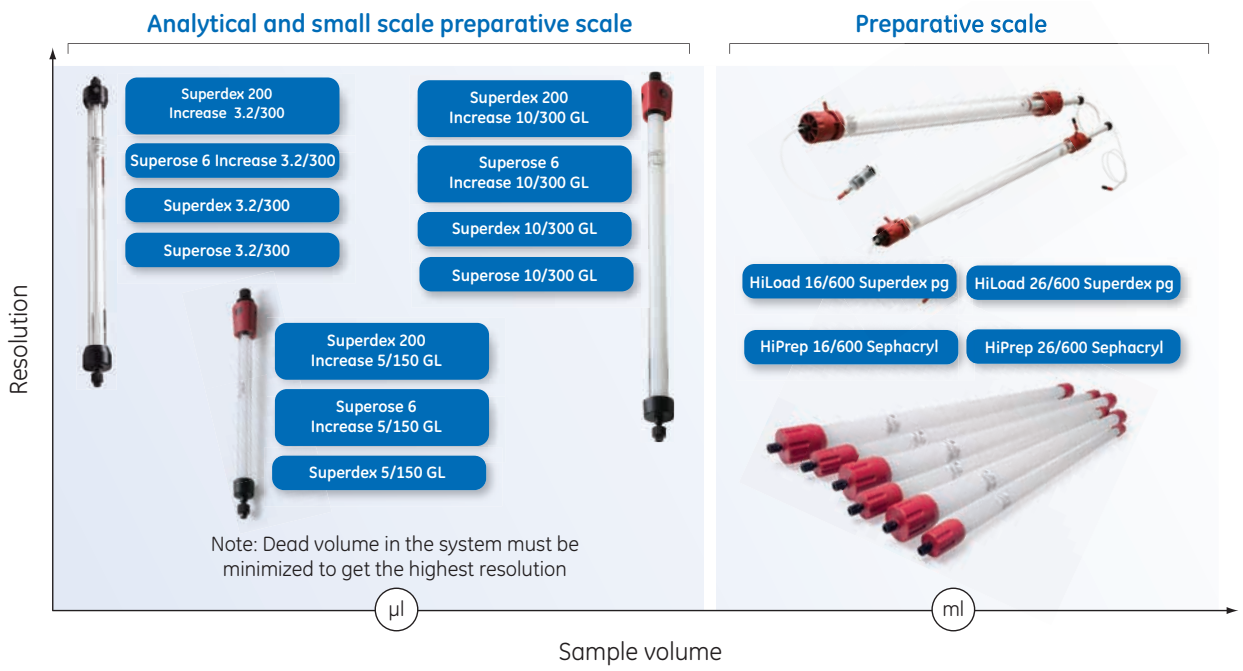


Fig 6. Schematic overview of resolution and sample volume for prepacked, high-resolution size exclusion chromatography columns.

Ordering information

Columns	Code number
Superdex Peptide 3.2/300	29-0362-31
Superdex Peptide 10/300 GL	17-5176-01
Superdex 75 3.2/300	29-0362-30
Superdex 75 10/300 GL	17-5174-01
Superdex 75 5/150 GL	28-9205-04
Superdex 200 Increase 3.2/300	28-9909-46
Superdex 200 Increase 10/300 GL	28-9909-44
Superdex 200 Increase 5/150 GL	28-9909-45
HiLoad 16/600 Superdex 30 pg	28-9893-31
HiLoad 26/600 Superdex 30 pg	28-9893-32
HiLoad 16/600 Superdex 75 pg	28-9893-33
HiLoad 26/600 Superdex 75 pg	28-9893-34
HiLoad 16/600 Superdex 200 pg	28-9893-35
HiLoad 26/600 Superdex 200 pg	28-9893-36
Superose 12 3.2/300	29-0362-25
Superose 12 10/300 GL	17-5173-01
Superose 6 Increase 3.2/300	29-0915-98
Superose 6 Increase 10/300 GL	29-0915-96
Superose 6 Increase 5/150 GL	29-0915-97
Superose 6 3.2/300*	29-0362-26
Superose 6 10/300 GL*	17-5172-01
HiPrep 16/60 Sephacryl S-100 HR	17-1165-01
HiPrep 26/60 Sephacryl S-100 HR	17-1194-01
HiPrep 16/60 Sephacryl S-200 HR	17-1166-01
HiPrep 26/60 Sephacryl S-200 HR	17-1195-01
HiPrep 16/60 Sephacryl S-300 HR	17-1167-01
HiPrep 26/60 Sephacryl S-300 HR	17-1196-01
HiPrep 16/60 Sephacryl S-400 HR	28-9356-04
HiPrep 26/60 Sephacryl S-400 HR	28-9356-05
HiPrep 16/60 Sephacryl S-500 HR	28-9356-06
HiPrep 26/60 Sephacryl S-500 HR	28-9356-07
HiTrap Desalting (1 × 5 ml)	29-0486-84
HiTrap Desalting (5 × 5 ml)	17-1408-01
HiTrap Desalting (100 × 5 ml)	11-0003-29
HiPrep 26/10 Desalting (1 × 53 ml)	17-5087-01
HiPrep 26/10 Desalting (4 × 53 ml)	17-5087-02
PD-10 Desalting Columns (30 pcs)	17-0851-01

* Superose 6 3.2/300 and 10/300 GL columns are to be replaced by Superose 6 Increase columns. Superose 6 columns will be available until December 31, 2016.

Media	Pack size	Code number
Superdex 30 prep grade	150 ml	17-0905-01
Superdex 75 prep grade	150 ml	17-1044-01
Superdex 200 prep grade	150 ml	17-1043-01
Superose 12 prep grade	125 ml	17-0536-01
Superose 6 prep grade	125 ml	17-0489-01
Sephacryl S-100 HR	150 ml	17-0612-10
	750 ml	17-0612-01
Sephacryl S-200 HR	150 ml	17-0584-10
	750 ml	17-0584-01
Sephacryl S-300 HR	150 ml	17-0599-10
	750 ml	17-0599-01
Sephacryl S-400 HR	150 ml	17-0609-10
	750 ml	17-0609-01
Sephacryl S-500 HR	150 m	17-0613-10
	750 ml	17-0613-01
Sephacryl S-1000 SF	750 ml	17-0476-01
Sephadex G-10	100 g	17-0010-01
	500 g	17-0010-02
Sephadex G-25 Superfine	100 g	17-0031-01
Sephadex G-25 Fine	100 g	17-0032-01
	500 g	17-0032-02
Sephadex G-25 Medium	100 g	17-0033-01
	500 g	17-0033-02
Sephadex G-50 Fine	100 g	17-0042-01
	500 g	17-0042-02
Sephadex LH-20	25 g	17-0090-10
	100 g	17-0090-01
	500 g	17-0090-02

Related products	
Gel Filtration Calibration Kit, LMW	28-4038-41
Gel Filtration Calibration Kit, HMW	28-4038-42
Handbook: Size exclusion chromatography, Principles and Methods	18-1022-18

Selection guide - SEC media

Start here

High resolution fractionation

Group separation

Preparative or Analytical high resolution (M_r 100-600 000)

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

Preparative Larger biomolecules (M_r 1000-10^8)

Desalting/Buffer exchange

In organic solvents

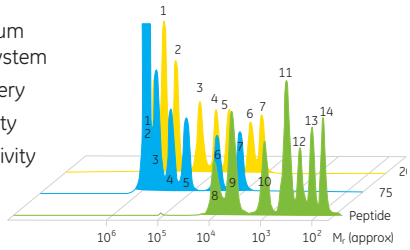
Superdex

High/medium pressure system

High recovery

High stability

High selectivity



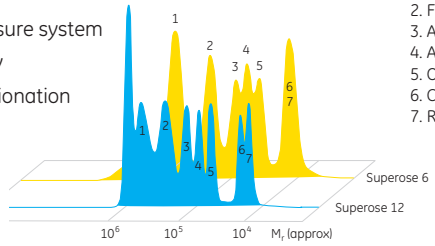
1. Thyroglobulin (M_r 669 000)
2. Ferritin (M_r 440 000)
3. Aldolase (M_r 158 000)
4. Albumin (M_r 67 000)
5. Ovalbumin (M_r 43 000)
6. Chymotrypsinogen A (M_r 25 000)
7. Ribonuclease A (M_r 13 700)
8. Cytochrome C (M_r 12 500)
9. Aprotinin (M_r 6500)
10. Gastrin I (M_r 2126)
11. Substance P (M_r 1348)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

Superose

Medium pressure system

High recovery

Wide M_r fractionation range



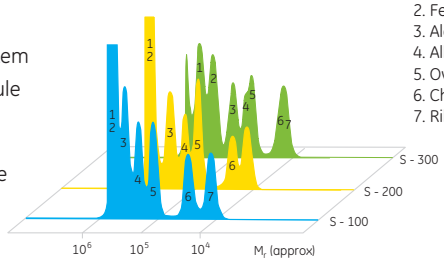
1. Thyroglobulin (M_r 669 000)
2. Ferritin (Mr_r440 000)
3. Aldolase (M_r 158 000)
4. Albumin (M_r 67 000)
5. Ovalbumin (M_r 43 000)
6. Chymotrypsinogen A (M_r 25 000)
7. Ribonuclease A (M_r 13 700)

Sephacryl

Low/medium pressure system

Macromolecule separation

Product line covering wide fractionation range

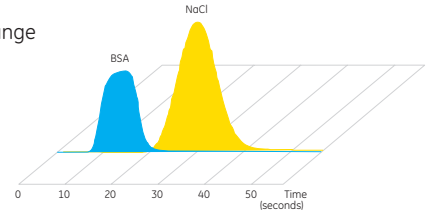


1. Thyroglobulin (M_r 669 000)
2. Ferritin (M_r 440 000)
3. Aldolase (M_r 158 000)
4. Albumin (M_r 67 000)
5. Ovalbumin (M_r 43 000)
6. Chymotrypsinogen A (M_r 25 000)
7. Ribonuclease A (M_r 13 700)

Sephadex

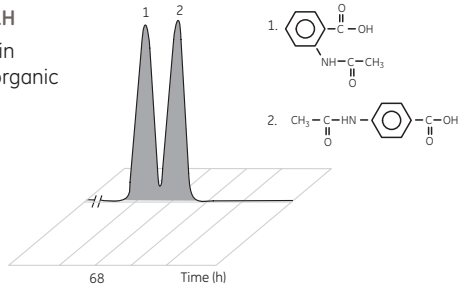
Desalting

Buffer exchange

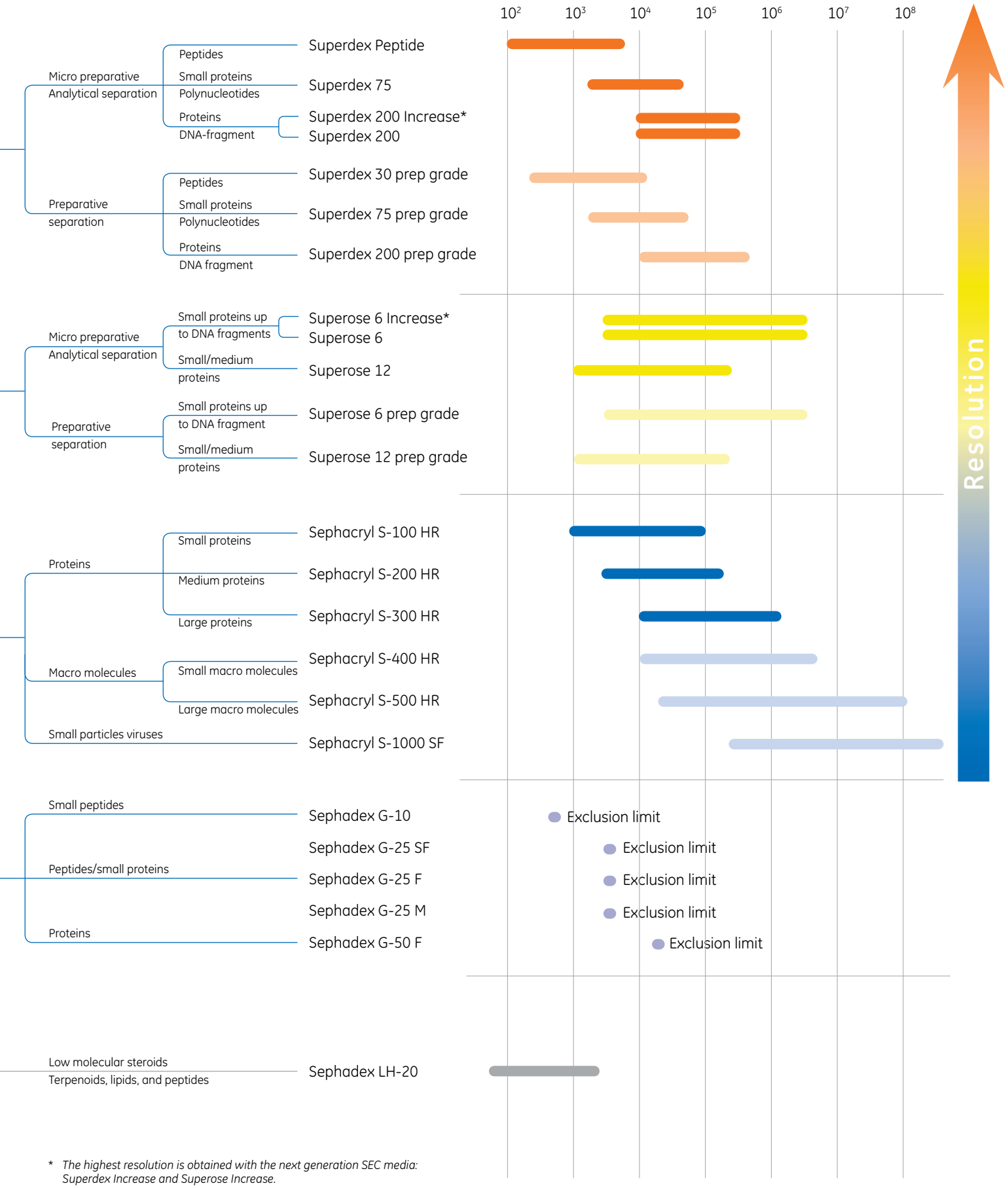


Sephadex LH

Separation in (nonpolar) organic solvent



Fractionation range
(globular proteins)



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

Product		Ordering information			Fractionation range globular proteins M_r (relative molecular weight)	Fractionation range dextrans M_r (relative molecular weight, Daltons)	Exclusion limit DNA (base pairs)	Average particle size (µm)	Column efficiency (N/m)	pH stability‡‡ (long term)
Prepacked column/Bulk media		Code number	Column dim. id × bed height (mm)	Pack size						
Group separation										
HiTrap™ Desalting HiTrap Desalting‡	B	17-1408-01 11-0003-29	16 × 25 16 × 25	5 100	1000 – 5000	100 – 5000	10	15–90	Not specified	2 to 13
HiPrep 26/10 Desalting HiPrep 26/10 Desalting	B	17-5087-01 17-5087-02	26 × 100 26 × 100	1 4	1000 – 5000	100 – 5000	10	20–80 (dry)	Not specified	2 to 13
PD-10 Desalting Columns††	B	17-0851-01	14.7 × 50	1	1000 – 5000	100 – 5000	10	86–258	Not specified	2 to 13
Sephadex G-10†	B	17-0010-01 17-0010-02	– –	100 g 500 g	> 700	> 700	2	40–120 (dry)	–	2 to 13
Sephadex G-25 Superfine†	B	17-0031-01	–	100 g	1000 – 5000	100 – 5000	10	20–50 (dry)	–	2 to 13
Sephadex G-25 Fine†	B	17-0032-01 17-0032-02	– –	100 g 500 g	1000 – 5000	100 – 5000	10	20–80 (dry)	–	2 to 13
Sephadex G-25 Medium†		17-0033-01 17-0033-02	– –	100 g 500 g	1000 – 5000	100 – 5000	10	50–150 (dry)	–	2 to 13
Sephadex G-50 Fine†		17-0042-01 17-0042-02	– –	100 g 500 g	1000 – 30 000	500 – 10 000	No data	20–80 (dry)	–	2 to 10
Sephadex LH-20†		17-0090-10 17-0090-01 17-0090-02	– – –	25 g 100 g 500 g	< 5000	No data	–	27–163 (dry)	–	2 to 11
High resolution fractionation										
Superdex Peptide 3.2/300 Superdex Peptide 10/300 GL		29-0362-31 17-5176-01	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
Superdex 75 3.2/300 Superdex 75 10/300 GL Superdex 75 5/150 GL		29-0362-30 17-5174-01 28-9205-04	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
Superdex 200 Increase 3.2/300		28-9909-46	3.2 × 300	1	10 000 – 600 000	1000 – 100 000		8.6	> 48 000	3 to 12
Superdex 200 Increase 10/300 GL Superdex 200 Increase 5/150 GL		28-9909-44 28-9909-45	10 × 300 5 × 150	1 1	10 000 – 600 000 10 000 – 600 000	1000 – 100 000 1000 – 100 000		8.6 8.6	> 48 000 > 42 000	3 to 12 3 to 12
Superdex 200 3.2/300* Superdex 200 10/300 GL* Superdex 200 5/150 GL*		29-0362-32 17-5175-01 28-9065-61	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	200 200 200	13 13 13	> 30 000 > 30 000 > 25 000	3 to 12 3 to 12 3 to 12
HiLoad 16/600 Superdex 30 pg HiLoad 26/600 Superdex 30 pg	B	28-9893-31 28-9893-32	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
HiLoad 16/600 Superdex 75 pg HiLoad 26/600 Superdex 75 pg	B	28-9893-33 28-9893-34	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
HiLoad 16/600 Superdex 200 pg HiLoad 26/600 Superdex 200 pg	B	28-9893-35 28-9893-36	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
Superdex 30 prep grade†	B	17-0905-01		150 ml	< 10 000	No data	No data	34	–	3 to 12
Superdex 75 prep grade†	B	17-1044-01		150 ml	3000 – 70 000	500 – 30 000	No data	34	–	3 to 12
Superdex 200 prep grade†	B	17-1043-01		150 ml	10 000 – 600 000	1000 – 100 000	No data	34	–	3 to 12
Superose 12 3.2/300 Superose 12 10/300 GL		29-0362-25 17-5173-01	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
Superose 6 Increase 3.2/300		29-0915-98	3.2 × 300	1	5 000 – 5 000 000	1 000 – 300 000		8.6	> 48 000	3 to 12
Superose 6 Increase 10/300 GL		29-0915-96	10 × 300	1	5 000 – 5 000 000	1 000 – 300 000		8.6	> 48 000	3 to 12
Superose 6 Increase 5/150 GL		29-0915-97	5 × 150	1	5 000 – 5 000 000	1 000 – 300 000		8.6	> 42 000	3 to 12
Superose 6 3.2/300 Superose 6 10/300 GL		29-0362-26 17-5172-01	3.2 × 300 10 × 300	1 1	5000 – 5 000 000 5000 – 5 000 000	No data No data	450 450	13 13	> 30 000 > 30 000	3 to 12 3 to 12
Superose 12 prep grade		17-0536-01	–	125 ml	1000 – 300 000	No data	150	30	–	3 to 12
Superose 6 prep grade		17-0489-01	–	125 ml	5000 – 5 000 000	No data	450	30	–	3 to 12
HiPrep 16/60 Sephacryl S-100 HR HiPrep 26/60 Sephacryl S-100 HR	B	17-1165-01 17-1194-01	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
HiPrep 16/60 Sephacryl S-200 HR HiPrep 26/60 Sephacryl S-200 HR	B	17-1166-01 17-1195-01	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
HiPrep 16/60 Sephacryl S-300 HR HiPrep 26/60 Sephacryl S-300 HR	B	17-1167-01 17-1196-01	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
Sephacryl S-100 HR†	B	17-0612-10 17-0612-01	– – 	150 ml 750 ml	1000 – 100 000	No data	No data	47	–	3 to 11
Sephacryl S-200 HR†	B	17-0584-10 17-0584-01	– – 	150 ml 750 ml	5000 – 250 000	1000 – 80 000	30	47	–	3 to 11
Sephacryl S-300 HR†	B	17-0599-10 17-0599-01	– – 	150 ml 750 ml	10 000 – 1 500 000	2000 – 400 000	118	47	–	3 to 11
HiPrep 16/60 Sephacryl S-400 HR HiPrep 26/60 Sephacryl S-400 HR	B	28-9356-04 28-9356-05	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
HiPrep 16/60 Sephacryl S-500 HR HiPrep 26/60 Sephacryl S-500 HR	B	28-9356-06 28-9356-07	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
Sephacryl S-400 HR†	B	17-0609-10 17-0609-01	– – 	150 ml 750 ml	20 000 – 8 000 000	10 000 – 2 000 000	271	47	–	3 to 11
Sephacryl S-500 HR†	B	17-0613-10 17-0613-01	– – 	150 ml 750 ml	No data	40 000 – 20 000 000	1078	47	–	3 to 11
Sephacryl S-1000 SF†		17-0476-01	–	750 ml	–	500 000 – 100 000 000	20 000	65	–	3 to 11

B BioProcess™ Media — Made for bioprocessing.

† Process scale quantities are avaible. Please contact GE Healthcare for further information.

‡ Pack size available by special order.

* Superdex 200 3.2/300, 5/150 GL and 10/300 GL columns are being replaced by Superdex 200 Increase columns. Superdex 200 columns will be available until December 31, 2015.

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
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
0.15/22	40 ml/min		2.5 to 15 ml	53	Fast and convenient group separation between high and low molecular weight substances
–	–		1.5 to 2.5 ml	8.3	Disposable column for group separation and buffer exchange
Can be calculated using Darcy´s Law	40 cm/h§		–	–	Fast and convenient group separation between peptides and low molecular weight substances
	20 cm/h§		–	–	Fast and convenient group separation between high and low molecular weight substances
	60 cm/h§		–	–	
	150 cm/h§		–	–	
	60 cm/h§		–	–	
0.15/22	30 cm/h§		–	–	Separation of natural products, such as steroids, terpenoids and lipids, in organic solvents
2.0/290	0.15 ml/min	0.05 ml/min	4 to 50 µl	2.4	Small-scale preparative and analytical high-resolving separation of peptides and other small biomolecules
1.8/260	1.2 ml/min	< 1 ml/min	25 to 500 µl	24	
2.4/350	0.1 ml/min	0.05 ml/min	4 to 50 µl	2.4	Small-scale preparative and analytical high-resolving separation of proteins, small proteins and polynucleotides, e.g. recombinant tagged proteins
1.8/260	1.5 ml/min	0.75 ml/min	25 to 500 µl	24	
1.8/260	0.7 ml/min	0.3 ml/min	4 to 50 µl	3	Rapid size analysis of protein homogeneity in screening experiments
3.0/435	0.15 ml/min	0.075 ml/min	4 to 50 µl	2.4	Small scale preparative purification and analysis of proteins, especially monoclonal antibodies when small sample and buffer consumption is important
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.
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
1.5/220	0.1 ml/min	0.05 ml/min	4 to 50 µl	2.4	
1.5/220	1.0 ml/min	0.75 ml/min	25 to 500 µl	24	
1.5/220	0.8 ml/min	0.3 ml/min	4 to 50 µl	3	
0.3/42	1.7 ml/min	1.0 ml/min	≤ 5 ml	120	Preparative separation of peptides and other small biomolecules
0.3/42	4.4 ml/min	2.6 ml/min	≤ 13 ml	320	
0.3/42	1.7 ml/min	1.0 ml/min	≤ 5 ml	120	Rapid, preparative separation of proteins, peptides, polynucleotides, and other biomolecules
0.3/42	4.4 ml/min	2.6 ml/min	≤ 13 ml	320	
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
0.3/42	4.4 ml/min	2.6 ml/min	≤ 13 ml	320	
0.3/42	90 cm/h§	10-50 cm/h	–	–	Preparative separation of peptides and other small biomolecules
0.3/42	90 cm/h§	10-50 cm/h	–	–	Rapid, preparative separation of proteins, peptides, polynucleotides, and other biomolecules
0.3/42	90 cm/h§	10-50 cm/h	–	–	Rapid, preparative separation of proteins, especially monoclonal antibodies, DNA fragments, and other biomolecules
2.4/350	0.1 ml/min	0.04 ml/min	4 to 50 µl	2.4	Small-scale preparative and analytical high-resolving separation of proteins, peptides, oligonucleotides, and polysaccharides
3/435	1.5 ml/min	0.5 ml/min	25 to 500 µl	24	
3.0/435	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
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
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
1.2/175	0.1 ml/min	0.04 ml/min	4 to 50 µl	2.4	
1.5/217	1.0 ml/min	0.5 ml/min	25 to 500 µl	24	
0.7/100	40 cm/h§	up to 40 cm/h		–	Preparative high-performance separation of proteins, peptides, oligonucleotides, and polysaccharides
0.4/58	40 cm/h§	up to 40 cm/h		–	Preparative high-performance separation of proteins, peptides, oligonucleotides, polysaccharides, and nucleic acids
0.15/22	1.0 ml/min	0.5 ml/min	≤ 5 ml	120	Preparative separation of proteins and peptides
0.15/22	2.7 ml/min	1.3 ml/min	≤ 13 ml	320	
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
0.15/22	2.7 ml/min	1.3 ml/min	≤ 13 ml	320	
0.15/22	1.0 ml/min	0.5 ml/min	≤ 5 ml	120	Preparative separation of proteins e.g., membrane proteins and antibodies
0.15/22	2.7 ml/min	1.3 ml/min	≤ 13 ml	320	
0.2/29	60 cm/h§	10-35 cm/h	–	–	Preparative separation of proteins and peptides
0.2/29	60 cm/h§	10-35 cm/h	–	–	Preparative separation of proteins e.g., small serum proteins such as albumin
0.2/29	60 cm/h§	10-35 cm/h	–	–	Preparative separation of proteins e.g., membrane proteins and antibodies
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
0.15/22	2.7 ml/min	1.3 ml/min	≤ 13 ml	320	
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
0.15/22	2.7 ml/min	1.3 ml/min	≤ 13 ml	320	
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
0.2/29	50 cm/h§	10-35 cm/h	–	–	Preparative separation of large macromolecules e.g., group separation of DNA restriction fragments
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

§ 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 (Code No. 18-3216-03) 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 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.

www.gelifesciences.com/protein-purification

GE Healthcare UK Limited, Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK.
GE Healthcare Bio-Scienes AB, Björgatan 30, 751 84 Uppsala, Sweden
GE Healthcare Europe, GmbH, Munzinger Strasse 5, D79111 Freiburg, Germany
GE Healthcare Bio-Sciences Corp., 800 Centennial Ave., P.O. Box 1327, Piscataway, NJ 08855-1327 USA
GE Healthcare Japan Corporation, Sanken Bldg., 3-25-1, Hyakunincho, Shinjuku-ku, Tokyo 169-0073, Japan

18-1124-19 AH 02/2015