

HiLoad 16/600 Superose 6 prep grade column

HiLoad™ 16/600 Superose™ 6 prep grade (pg) prepacked column (Fig 1) is designed for purification of large proteins and protein complexes by size exclusion chromatography (SEC) in sample volumes up to 5 mL. The column is packed with Superose 6 prep grade chromatography resin having a fractionation range suitable to separate biomolecules with molecular weights (M_r) from ~ 5000 to 5 000 000 by size exclusion chromatography (SEC). This very wide fractionation range also makes the column suitable for purification of membrane proteins and other macromolecules.

HiLoad 16/600 Superose 6 pg column offers:

- Convenient prepacked column format that removes the need for self-packing and enables highly reproducible protein separations
- High-resolution separation of protein complexes and other large biomolecules for high protein purity
- High chemical stability enabling thorough cleaning for long column lifetime and minimal carry-over
- Easy connection to ÄKTA™ and other liquid chromatography systems

HiLoad 16/600 Superose 6 pg column is an empty XK column that has been expertly packed and individually tested. The combination of prepacked convenience and reproducibility makes HiLoad 16/600 Superose 6 pg column a confident choice for fast, high-resolution SEC at preparative scale. At maximum recommended sample load (5 mL), up to about 250 mg of proteins can be purified.

The column operates with a wide variety of equipment, including simple pump-based configurations and ÄKTA chromatography systems.

Resin characteristics

The chromatography resin in the column is Superose 6 prep grade and this is based on cross-linked agarose. The size and the distribution of the particles allow high flow, high efficiency, and good capacity. Further characteristics of Superose 6 prep grade resin are shown in Table 1.



Fig 1. HiLoad 16/600 Superose 6 pg column brings convenience and high resolution to size exclusion chromatography (also known as gel filtration).

Table 1. Main characteristics of Superose 6 prep grade resin

Matrix	Composite of cross-linked agarose
Particle size, $d_{50\mu}$ ¹	30 ± 10 µm
Fractionation range	M_r ~ 5 × 10 ³ to 5 × 10 ⁶ (globular proteins)
Exclusion limit (M_r)	~ 4 × 10 ⁷ (globular proteins)
pH stability	
operational ²	3 to 12
cleaning-in-place (CIP) ³	1 to 14
Temperature	
operational	4°C to 40°C
storage	4°C to 30°C

¹ Median particle size of the cumulative volume distribution.

² pH range where resin can be operated without significant change in function.

³ pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

Column characteristics

Each HiLoad column has a precision borosilicate glass tube and a fitted thermostatic jacket. Dead volumes make up less than 0.1% of the total column volume, keeping sample dilution and band broadening to a minimum.

Valco™ fittings (1/16") are standard and provide easy and direct connection to ÄKTA chromatography systems.

Every prepacked HiLoad column is tested for column efficiency (number of theoretical plates per meter, N/m), asymmetry factor (A_s), and bed height (mm). These stringent control measures ensure that HiLoad columns give reproducible results time after time. For column characteristics, see Table 2.

Selectivity of Superose 6 prep grade resin and other SEC resins from Cytiva

The distribution coefficient, K_{av} , is related to the size of a molecule. Molecules of similar shape and density demonstrate a sigmoidal relationship between their K_{av} values and the logarithms of their relative molecular weights (M_r). The relationship between $\log M_r$ and K_{av} is virtually linear over a considerable range. The practical working range of K_{av} is approximately 0.1 to 0.9.

SEC resins should be selected so that the important components are found in the middle of the selectivity curve with minimum peak broadening or dilution and minimum time on the column. A steeper selectivity curve means an improved separation (high selectivity) but a small separation range.

Superose 6 prep grade compared with Superose 6 Increase resin

Another Cytiva resin used for separation of large biomolecules is Superose 6 Increase. This resin is used for purification of small sample volumes up to 0.5 mL. Superose 6 prep grade and Superose 6 Increase resins have a similar fractionation range of 5000 to 5 000 000 (Fig 2). The offset parallel lines in Figure 2 are a result of the differences in pore size distribution of the resins, that is, the selectivity, of the two resins. In practice, the same molecules may be separated on Superose 6 prep grade and Superose 6 Increase, but they elute at slightly different column volumes. The resolution with Superose 6 Increase will be higher because of the much smaller particle size (~ 9 μm) compared with Superose 6 prep grade (~ 30 μm).

Table 2. Characteristics of HiLoad 16/600 Superose 6 prep grade column

Bed dimensions, diameter × height (mm)	Approximate bed volume (mL)	Column efficiency, (N/m)	Recommended sample volume (mL)	Recommended operating flow rate, H ₂ O at 25°C (mL/min) ¹	Maximum operating flow rate, H ₂ O at 25°C (mL/min) ¹	Maximum operating pressure (MPa)
16 × 600	120 to 124	> 10 000	Up to 5	1.0	1.6	0.3 (3.0 bar, 43.5 psi)

¹ Note: Flow rate needs to be decreased when working at low temperature or with viscous solutions. See product instruction for more details.

Table 3. Fractionation ranges for some SEC resins from Cytiva

	Superdex 30 prep grade	Superdex 75 prep grade	Superdex 200 prep grade	Superose 6 prep grade	Superose 6 Increase
Fractionation range (M_r) globular proteins	< 10 000	~ 3000 to 70 000	~ 10 000 to 600 000	~ 5000 to 5 000 000 ¹	~ 5000 to 5 000 000 ¹

¹ The same molecules may be separated on Superose 6 prep grade and Superose 6 Increase, but the resolution will be higher with Superose 6 Increase because of the smaller particle size.

Superose 6 prep grade compared with Superdex prep grade resins

The fractionation range of Superose 6 prep grade resin is complementary to that of Cytiva's Superdex™ 75 prep grade and Superdex 200 prep grade resins (Fig 3); Superdex 75 prep grade gives excellent resolution for biomolecules from M_r 3000 to 70 000 while Superdex 200 prep grade allows fractionation in the M_r 10 000 to 600 000 range. Superdex 30 prep grade is also available prepacked in HiLoad columns with a fractionation range up to M_r 10 000, which is suitable for purification of peptides and other small biomolecules.

Fractionation ranges for SEC resins from Cytiva are summarized in Table 3.

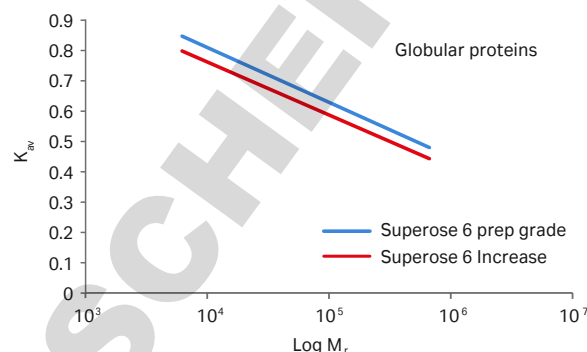


Fig 2. Selectivity curve for Superose 6 prep grade and Superose 6 Increase.

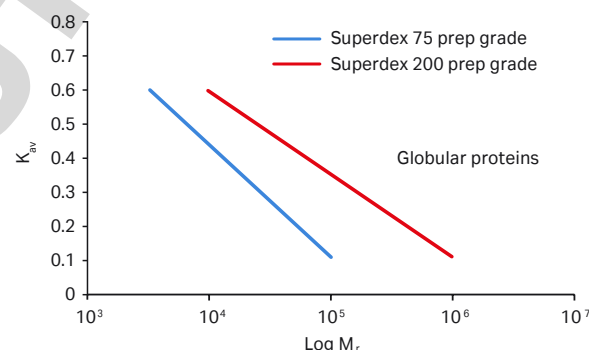


Fig 3. Selectivity curve for Superdex 75 prep grade and Superdex 200 prep grade.

Chemical stability

Superose 6 prep grade is resistant to commonly used solutions in size exclusion chromatography, including 8 M urea, 6 M guanidine HCl, and 30% acetonitrile. A packed column should be used in 4°C to 40°C. Superose 6 prep grade is stable for operational use in the pH range 3 to 12. For CIP (cleaning-in-place) the pH range could be extended to 1 to 14. High concentrations > 1 M formic acid should not be used. Detergents, nonionic or ionic such as SDS may be used. Limited degradation of the polysaccharide chains may occur under oxidizing conditions.

Separation of standard proteins on Superose 6 pg and Superose 6 Increase columns

HiLoad 16/600 Superose 6 pg column is suitable for separation of large proteins and proteins complexes for samples from 0.5 to 5 mL. Superose 6 Increase column on the other hand enables separation of samples for large proteins and protein complexes, but for smaller volumes < 0.5 mL.

In Figure 4 a typical separation on a HiLoad 16/600 Superose 6 pg column of standard proteins is shown. In Figure 5, the same set of standard proteins is separated on a Superose 6 Increase 10/300 GL column for comparison. When scaling up from the 24 mL Superose 6 Increase column to the 120 mL HiLoad 16/600 Superose 6 pg column, a decrease in resolution is expected due to the smaller particle size of Superose 6 Increase. The resolution between the peaks of the six separated proteins on the two columns, respectively is shown in Table 4.

Column: HiLoad 16/600 Superose 6 pg
Sample: 1. Thyroglobulin, 3 mg/mL
 2. Ferritin, 0.3 mg/mL
 3. Aldolase, 3 mg/mL
 4. Ovalbumin, 3 mg/mL
 5. Ribonuclease A, 3 mg/mL
 6. Aprotinin, 1 mg/mL
Sample volume: 500 µL
Eluent: 10 mM phosphate buffer, 140 mM NaCl, pH 7.4
Flow rate: 1.27 mL/min
Detection: 280 nm
System: ÄKTA pure 25

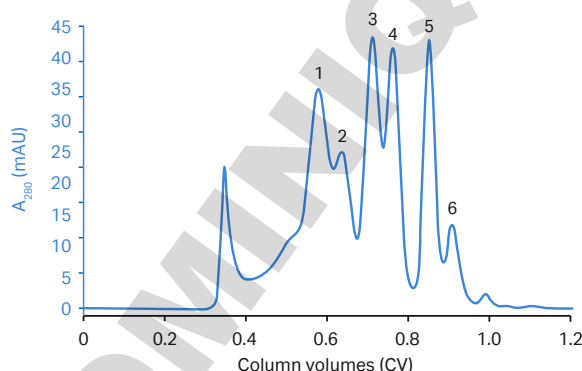


Fig 4. Standard proteins separated on HiLoad 16/600 Superose 6 pg.

Column: Superose 6 Increase 10/300 GL
Sample: 1. Thyroglobulin, 3 mg/mL
 2. Ferritin, 0.3 mg/mL
 3. Aldolase, 3 mg/mL
 4. Ovalbumin, 3 mg/mL
 5. Ribonuclease A, 3 mg/mL
 6. Aprotinin, 1 mg/mL
Sample volume: 100 µL
Eluent: 10 mM phosphate buffer, 140 mM NaCl pH 7.4
Flow rate: 0.5 mL/min
Detection: 280 nm
System: ÄKTA micro

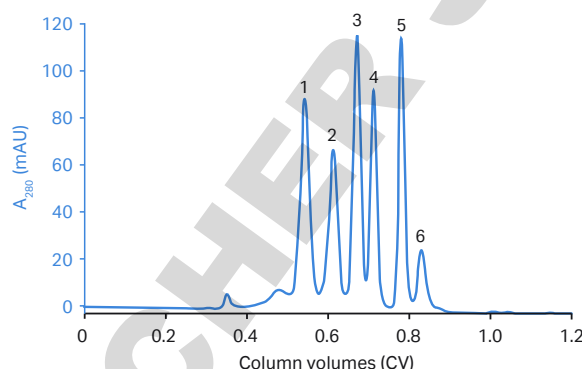


Fig 5. Standard proteins separated on Superose 6 Increase 10/300 GL.

Table 4. Resolution (R_s) between the peaks of six proteins separated on HiLoad 16/600 Superose 6 prep grade and Superose 6 Increase 10/300 GL columns, respectively

Proteins	HiLoad 16/600 Superose 6 prep grade, R_s	Superose 6 Increase 10/300 GL, R_s
Thyroglobulin/ferritin	0.56	1.63
Ferritin/aldolase	0.90	1.55
Aldolase/ovalbumin	0.64	1.26
Ovalbumin/ ribonuclease A	1.44	2.48
Ribonuclease A/ aprotinin	0.91	1.62

Ordering information

Product	Quantity	Product code
HiLoad 16/600 Superose 6 prep grade	1 × 120 mL	29323952
Superose 6 prep grade	125 mL	17048901

Related products

Size exclusion chromatography columns

HiLoad 16/600 Superdex 30 prep grade	1 × 120 mL	28989331
HiLoad 26/600 Superdex 30 prep grade	1 × 320 mL	28989332
HiLoad 16/600 Superdex 75 prep grade	1 × 120 mL	28989333
HiLoad 26/600 Superdex 75 prep grade	1 × 320 mL	28989334
HiLoad 16/600 Superdex 200 prep grade	1 × 120 mL	28989335
HiLoad 26/600 Superdex 200 prep grade	1 × 130 mL	28989336
Superose 6 Increase 10/300 GL	1 × 24 mL	29091596

HiLoad column accessories

Accessory kit XK 16 ¹	1	28989978
Support screen XK 16	5	19065101
Net ring (10 µm) XK 16	5	18876101
O-ring XK 16	5	19016301
Stop plug female, 1/16"	5	11000464
HiTrap/HiPrep 1/16" male connector for ÄKTA	8	28401081
Transport device	1	18117643

Whatman™ syringe filters for ÄKTA systems

Protein Prep filter for ÄKTA systems 0.45 µm, 30 mm	150	10463033
Protein Prep filter for ÄKTA systems 0.2 µm, 30 mm	150	10463043

¹ Accessory kit XK 16 is suitable for repacking purposes and contains: 2 support screens, 5 net rings, 2 O-rings, 2 stop plugs, 10 HiTrap/HiPrep 1/16" male connectors for ÄKTA systems, and 1 tool for dismantling.

Related literature

Size exclusion chromatography, principles and methods, Handbook	18102218
Size exclusion chromatography columns and resins, Selection guide	18112419
Prepacked chromatography columns for ÄKTA systems, Selection guide	28931778
Superdex prep grade and prepacked HiLoad columns, Data file	18110052

cytiva.com/protein-purification

Cytiva and the Drop logo are trademarks of Global Life Sciences IP Holdco LLC or an affiliate. ÄKTA, HiTrap, Superdex, Superose and Whatman are trademarks of Global Life Sciences Solutions USA LLC or an affiliate doing business as Cytiva.

Valco is a trademark of Valco Instruments Company, Inc. All other third-party trademarks are the property of their respective owners.

© 2020 Cytiva

All goods and services are sold subject to the terms and conditions of sale of the supplying company operating within the Cytiva business. A copy of those terms and conditions is available on request. Contact your local Cytiva representative for the most current information.

For local office contact information, visit cytiva.com/contact

CY13872-01Sep20-DF

