Superdex 200 prep grade – unmatched resolution and speed

Ever since the introduction of Sephadex™, gel filtration has occupied a key position in the purification of biomolecules. In gel filtration, biomolecules in solution are separated according to differences in sizes, as they pass through a column packed with a gel matrix. Gel filtration is a mild separation technique that can be performed under a wide range of conditions, according to the requirements of the specific biomolecules.

An inherent problem, when designing a gel filtration matrix, is to combine a controlled range of pore size with chemical and physical stability, and inertness.

Sephadex has been the standard to which many new gel filtration media have been compared. Sephadex is inert and has excellent selectivity due to its pore size distribution, but it is not physically strong.

Superdex™, which is a composite matrix of dextran and agarose, combines the steep selectivity curve characteristic of Sephadex with the physical and chemical stability of highly cross-linked agarose, see Figure 1. As a result, Superdex 200 prep grade enables high resolution to be obtained even at high flow rates, as will be illustrated below. Superdex 200 prep grade has a narrow particle size distribution (average 34 µm) and is optimized for preparative gel filtration.

Resolution of Superdex 200 prep grade compared to Sephadex G-200

Mouse monoclonal cell supernatant, IgG_1, was applied to a column packed with Superdex 200 prep grade and a column packed with Sephadex G-200. The two columns were run at the maximum recommended flow rates for each medium. Fractions were collected and the purity was checked with SDS-PAGE.

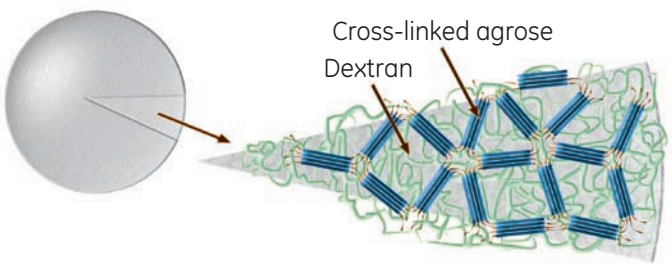


Fig 1. Hypothetical view of a section through a bead from Superdex 200 prep grade.

The higher resolution on Superdex, compared to Sephadex, is confirmed by the chromatograms and SDS-PAGE, see Figures 2 and 3.

Time savings

Since a rigid matrix can be used at a much higher flow rate than a softer matrix, a typical run on a Superdex 200 prep grade column will take less than two hours while a typical run on Sephadex G-200 will last overnight, see Figure 2.

The time required for performing a separation on Sephadex includes, apart from sample preparation, also preparation of the medium (swelling, degassing, packing, equilibration, and checking the bed) before a result is obtained. The difference in time required is illustrated in Table 1 and Figure 4. Using a column prepacked with Superdex, purified sample will be obtained after 3 hours and 20 minutes, while it will be obtained after 56 hours when using an XK 16/70 column packed with Sephadex G-200. The difference in the total time is striking.

Scale-up

Superdex 200 prep grade can easily be scaled up with the same performance, as illustrated by Figures 5A and 5B.

Columns: A) Sephadex G-200 packed in XK 16/70 column
B) HiLoad 16/60 Superdex 200 pg
Column volumes, CV: Approx. 120 ml
Sample: Mouse monoclonal cell supernatant, IgG_1
Sample pretreatment: Concentration approx. 40 × in Amicon™ concentration cell, PM10 filter
Sample volume: 1.2 ml (1% × CV)
Buffer: 50 mM monosodium phosphate, 0.15 M NaCl, pH 7.0
Flow rate: A) 0.2 ml/min (6 cm/h)
B) 1.6 ml/min (50 cm/h)
System: FPLC™ System with FPLCdirector™

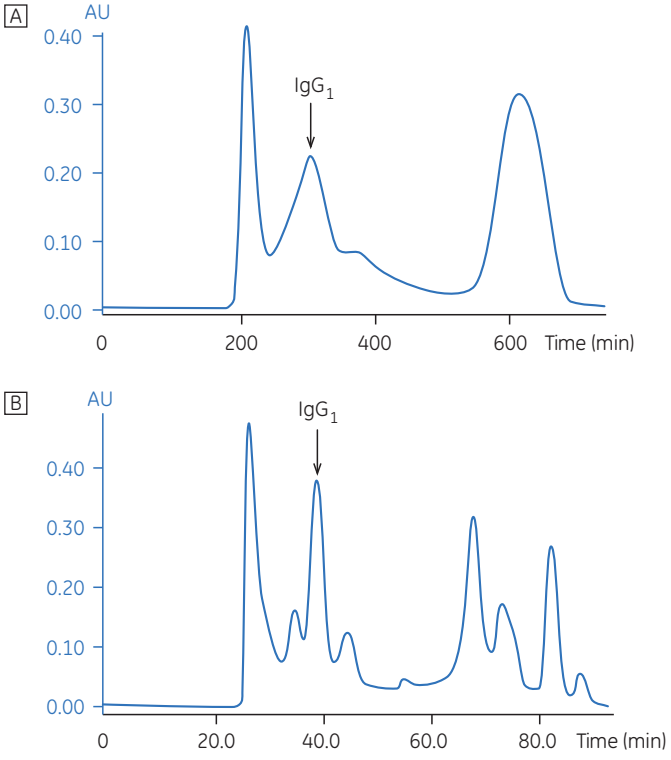
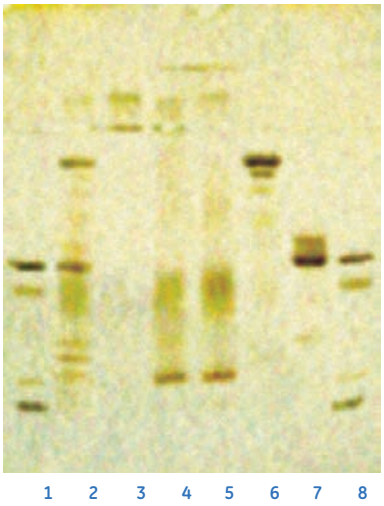


Fig 2. Chromatograms showing purification of IgG_1 from mouse monoclonal cell supernatant using **(A)** Sephadex G-200 and **(B)** Superdex 200 prep grade.

Purity check with SDS-PAGE: PhastGel™ Gradient 10–15
Buffer: PhastGel Buffer SDS Strips
Sample volumes: 1 µl
Standard: LMW-SDS Marker Kit
Staining: Silver, according to the manufacturer's protocol
System: PhastSystem™



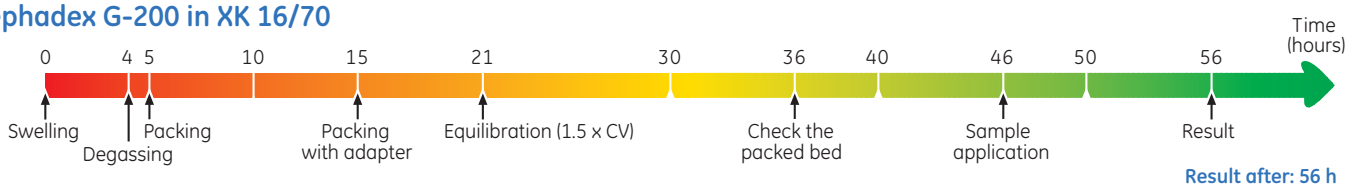
Lane 1: LMW markers
Lane 2: Starting material, cell culture supernatant including mouse IgG_1
Lane 3: Fraction 9
Lane 4: Fraction 14
Lane 5: Fraction 22
Lane 6: Fraction 29
Lane 7: Fraction 38
Lane 8: LMW markers

Fig 3. Purity check using SDS-PAGE and silver staining of the fractions from Superdex 200 prep grade (Fig 2B).

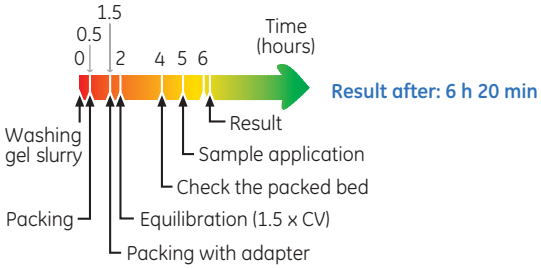
Table 1. Time table for purification using Sephadex G-200 and Superdex 200 prep grade. The numbers indicate the time, in hours, required for each step in the purification protocol

	Sephadex G-200, packed in an XK 16/70 (hours)	Superdex 200 prep grade, packed in an XK 16/70 (hours)	HiLoad 16/60 Superdex 200 pg, prepacked column (hours)
Swelling	4	not required	not required
Degassing	1	0.5	not required
Packing	16	1.5	not required
Equilibration	15	2	2
Checking the packed bed	10	1	not required
Sample application & elution	10	1.3	1.3
Total time	56	6.3	3.3

Sephadex G-200 in XK 16/70



Superdex 200 prep grade in XK 16/70



HiLoad 16/60 Superdex 200 pg

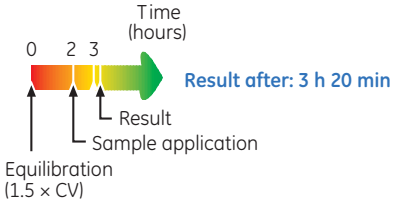


Fig 4. Time lines for performing a fractionation by gel filtration on Sephadex G-200, Superdex 200 prep grade, and HiLoad 16/60 Superdex 200 pg.

Columns:	HiLoad Superdex 200 pg
Column volumes, CV:	A) Approx. 120 ml (16/60) B) Approx. 320 ml (26/60)
Sample:	Mouse monoclonal cell supernatant, IgG_{2b}, incl. 1% fetal calf serum
Sample pretreatment:	Concentration approx. 40 × in Amicon concentration cell
Sample volume:	A) 1.2 ml B) 3.2 ml
Buffer:	50 mM monosodium phosphate, 0.15 NaCl, pH 7.0
Flow rate:	A) 1.6 ml/min (50 cm/h), (maximum recommended flow rate) B) 4.4 ml/min (50 cm/h), (maximum recommended flow rate)
System:	FPLC System with FPLCdirector

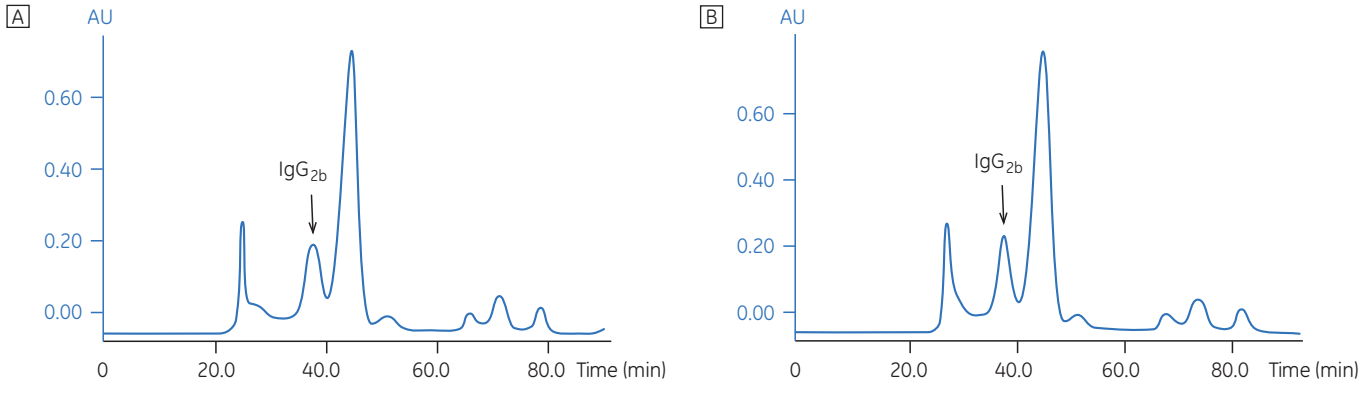


Fig 5. Purification of mouse monoclonal cell supernatant using **(A)** HiLoad 16/60 Superdex 200 pg, 120 ml, and **(B)** HiLoad 26/60 Superdex 200 pg, 320 ml.

Conclusion

Superdex 200 prep grade provides higher resolution and gives results faster than Sephadex G-200. Even greater time savings can be achieved by using prepacked HiLoad™ columns, with the additional assurance of optimal packing and operation efficiency.

Superdex is available with a range of selectivities and particle sizes, designed to work over different fractionation ranges and scales from analytical to full production. Superdex 200 prep grade, with a fractionation range between 10 000 and 600 000 is well suited to the separation of larger proteins including monoclonal antibodies.

Together, these properties make Superdex 200 prep grade the first choice in gel filtration media for all applications from laboratory to process scale.

Ordering information

Products	Quantity	Code no.
Prepacked columns		
HiLoad 16/60 Superdex 200 pg	1 × 120 ml	17-1069-01
HiLoad 26/60 Superdex 200 pg	1 × 320 ml	17-1071-01
Bulk media		
Superdex 200 prep grade	150 ml	17-1043-01
Superdex 200 prep grade	25 ml	17-1043-10
Empty lab-scale columns		
XK 16/70 column	1	18-8775-01
XK 26/70 column	1	18-8769-01
XK 16/40 column	1	18-8774-01

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GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

GE Healthcare Europe GmbH
Munzinger Strasse 5, D-79111 Freiburg, Germany

GE Healthcare UK Ltd
Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

GE Healthcare Bio-Sciences Corp
800 Centennial Avenue, P.O. Box 1327, Piscataway,
NJ 08855-1327, USA

GE Healthcare Bio-Sciences KK
Sanken Bldg. 3-25-1, Hyakunincho, Shinjuku-ku,
Tokyo 169-0073, Japan

Asia Pacific T +85 65 62751830 F +85 65 62751829 • Australasia T +61 2 8820 8299 F +61 2 8820 8200 • Austria T 01 /57606 1613 F 01 /57606 1614 • Belgium T 0800 73 890 F 02 416 8206 • Canada T 1 800 463 5800 F 1 800 567 1008 • Central & East Europe T +43 1 972 720 F +43 1 972 722 750 • Denmark T +45 70 25 24 50 F +45 45 16 2424 • Eire T 1 800 709992 F +44 1494 542010 • Finland & Baltics T +358 9 512 3940 F +358 9 512 39439 • France T 01 69 35 67 00 F 01 69 41 98 77 Germany T 0800 9080 711 F 0800 9080 712 • Greater China T +852 2100 6300 F +852 2100 6338 • Italy T 02 26001 320 F 02 26001 399 • Japan T 81 3 5331 9336 F 81 3 5331 9370 • Korea T 82 2 6201 3700 F 82 2 6201 3803 • Latin America T +55 11 3933 7300 F +55 11 3933 7304 • Middle East & Africa T +30 210 96 00 687 F +30 210 96 00 693 • Netherlands T 0800-82 82 82 1 F 0800-82 82 82 4 • Norway T +47 815 65 777 F +47 815 65 666 • Portugal T 21 417 7035 F 21 417 3184 • Russia, CIS & NIS T +7 495 956 5177 F +7 495 956 5176 • Spain T 902 11 72 65 F 935 94 49 65 • Sweden T 018 612 1900 F 018 612 1910 • Switzerland T 0848 8028 10 F 0848 8028 11 • UK T 0800 515 313 F 0800 616 927 • USA T +1 800 526 3593 F +1 877 295 8102

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