

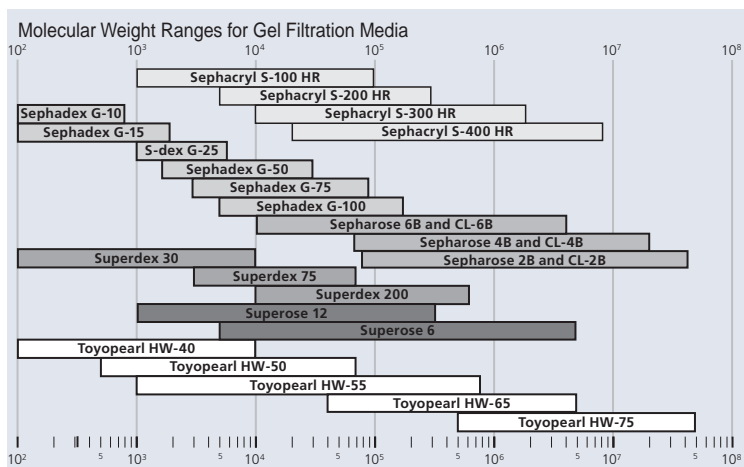
Gel Filtration Chromatography

In gel filtration and gel permeation chromatography – also known as size exclusion chromatography – separation is based on differences in the size and/or shape of the analyte molecules, which governs the analytes' access to the pore volume inside the column packing particles. The exclusion limit of a size exclusion packing indicates the molecular weight, for a particular polymer type, above which analytes are fully excluded from entering the pores and thus will not be separated. According to their size, smaller analytes have partial to complete access to the pore volume. Among the analytes that partially or fully enter the pore volume, larger molecules with less access to the pore volume elute first, while the smallest molecules elute last. The fractionation range identifies the molecular weight range from the largest molecule that is fully included to the smallest molecule that is fully excluded.

The Supelco® Media Store (sigma-aldrich.com/lc-media) offers gel filtration media for low and medium pressure liquid chromatography. Packing materials are supplied with instructions for packing columns for use with gravity feed or low to medium pressure pumps.

Media selection charts in this guide contain pertinent information about each gel filtration packing material. Organized alphabetically by medium name, the charts list chemical composition, exclusion limits, pH and pressure stability, and particle size ranges. Key properties, limitations, and potential applications also are listed.

Refer to the adsorption media listings in the Supelco catalog for Sephadex LH-20, which is used for purifying small molecular weight compounds by gel permeation and partition chromatography. Hydroxyalkoxypropyl dextran is also listed with the adsorption media.





Gel Filtration Media

Medium	Fractionation Ranges (kDa, proteins)	Properties/Limitations	Potential Applications
Sephacryl®			
Matrix: acrylic (dextran/bisacrylamide copolymer)			
25-75 µm	S-100 HR: 1-100	Clean with 0.2 M NaOH, 0.1 M HCl, 1.0 M HOAc	S-100 HR: peptides, small proteins
S-1000: 40-105 µm	S-200 HR: 5-250	Compatible with detergents, chaotropic salts, dissociating agents	S-200 HR: proteins, some small serum proteins (e.g., albumin)
0.2 MPa (2.6 x 30 cm column)	S-300 HR: 10-1500	Sterilize by autoclave	S-300 HR: membrane & serum proteins, monoclonal antibodies
pH range: 2-11	S-400 HR: 20-8000	Compatible with organic solvents	S-400 HR: large proteins & macromolecules with extended structures (e.g., proteoglycans, liposomes)
	S-500 HR, S-1000: ND	Scaleable Good recoveries Stable bed volume	S-500 HR: large macromolecules, small particles (e.g., plasmids)
Sephadex®			
Matrix: dextran (crosslinked with epichlorohydrin)			
C: 100-300 µm	G-10: <0.7	Classic support	G-10: very low MW substances; desalting; buffer exchange;
M: 50-150 µm	G-15: <1.5	Swelling 2-40×	peptides
F: 20-80 µm	G-25: 1-5	SF grade for best resolution, low flow rate	G-15: low MW compounds; interact with aromatic compounds; desalting; buffer exchange; peptides
SF: 10-40 µm	G-50: 1.5-30	F grade for prep lab work	G-25: medium grade available in packed PD-10 columns for rapid, routine desalting; interact with aromatic compounds;
SF: 0.016-0.160 MPa (2.6 x 30 cm column)	G-75: 3-80	M grade for standard lab work	small peptides & proteins; buffer exchange
pH range: 2-13 (< G-25) 2-10 (> G-25)	G-100: 4-150	C grade for very crude samples Can be autoclaved at 120 °C Compatible with detergents, chaotropic salts, dissociating agents Clean with 0.2 M NaOH Ideal for MW determinations Interact with aromatic compounds	G-100: very low nonspecific interactions
Sepharose®			
Matrix: agarose			
6B: 45-165 µm	6B: 10-4000	Clean with 0.5 M NaOH	2B: DNA-protein complexes; viruses; asymmetric molecules; affinity support
4B: 45-165 µm	4B: 60-20,000	Good recoveries	4B: tRNAs; membrane proteins; polysaccharides; affinity support
2B: 60-200 µm	2B: 70-40,000	Broad fractionation range	6B: polio virus purification; proteins; polysaccharides;
0.004-0.02 MPa (2.6 x 30 cm column)		Temperature range: 4-40 °C Cannot be autoclaved	
pH range: 4-9		Incompatible with oxidizing agents, chaotropic salts, dissociating agents	

Gel Filtration Media

Medium	Fractionation Ranges (kDa, proteins)	Properties/Limitations	Potential Applications
Sephacrose® CL			
Matrix: agarose (Sephacrose reacted with 2,3-dibromopropanol)			
6B: 45-165 µm	6B: 10-4000	Stable in chaotropic salts, dissociating agents	2B: very high MW molecules (e.g., nucleic acids); polysaccharides; cell particles; viruses
4B: 45-165 µm	4B: 60-20,000	Flow 2× that of Sepharose	4B: vesicles; membrane proteins; polysaccharides; affinity support
2B: 60-200 µm	2B: 70-40,000	Sterilize by autoclave	6B: viruses; proteins; polysaccharides; affinity support.
0.005-0.02 MPa		Easy switch to organic solvents	
(2.6 x 30 cm column)		Good recoveries	
pH range: 3-14		Broad fractionation range	
		Ideal for long-term routine applications	
		Do not use with oxidizing agents	
Superdex®			
Matrix: dextran (covalently bonded to agarose)			
24-44 µm	30: <10	Newest member of GFC family	30: peptides; oligosaccharides; small proteins. Selectivity between Sephadex® G-25 & G-50
0.5 MPa	75: 3-70	Clean with 1 M NaOH, 0.1 M HCl, 1.0 M HOAc	75: high resolution prep separations; wide range of recombinant DNA products. Selectivity similar to Sephadex G-75
pH range: 3-12	200: 10-600	Can be used with detergents, chaotropic salts, dissociating agents	200: monoclonal antibodies; high resolution prep separations; contaminants of lower MW (albumin, transferrin); good when protein MW is unknown. Selectivity similar to Sephadex G-200
		Sterilize by autoclave	
		Compatible with organic solvents	
		Scaleable	
		High recoveries	
		Highest selectivity	
Superose®			
Matrix: agarose (highly crosslinked)			
20-40 µm	12: 1-300	Clean with 0.2 M NaOH, 0.01 M HCl, 1.0 M HOAc	Preparative work; proteins; DNA fragments; polysaccharides
12: 0.7 MPa	6: 5-5000	Compatible with detergents, chaotropic salts, dissociating agents	
6: 0.4 MPa		Sterilize by autoclave	
(1.6 x 50 cm column)		Compatible with organic solvents	
pH range: 2-14		FPLC-HiLoad	
		Some hydrophobic interactions	
Toyopearl® HW			
Matrix: methacrylate (ethylene glycol/methacrylate copolymer)			
C: 50-100 µm	40: <10	High mechanical strength (5-7 bar)	HW-40: very effective with organics; phthalates; cyclodextrins; low MW nucleosides/nucleotides; peptide digests
F: 30-60 µm	50: 500-80	Stable in organics	HW-50: medium MW molecules; peptides
S: 20-40 µm	55: 1-700	Surfactant, dissociating agent, acid/base cleaning	HW-55: medium MW molecules; RNase digest; tissue plasminogen activator; RNA aggregates; glycoproteins
0.7 M Pa	65: 40-5000	Autoclavable	HW-65: polysaccharides; mRNA; collagenous proteins
pH range: 2-12	75: 500-50,000	No saccharide derivative leaching	HW-75: large MW molecules; amylose subfractions; plasmid DNA; replace ultracentrifugation steps
		High flow rate	
		Useful for very hydrophobic proteins	
		Stable bed volume	
		S: high resolution, high pressure drop, low flow rate	
		C: low resolution, low pressure, high flow rate	

80 cm H₂O = 0.8 bar = 11.6 psi = 0.08 MPa





Recommended Reading (for Gel Filtration Chromatography)

Gel Filtration: Principles and Methods (5th Edition)

Pharmacia Biotech, 1991, 102 pp.

Since the introduction of Sephadex®, 30 years ago, gel filtration has had a key role in the purification of thousands of biological macromolecules. The enduring value of Sephadex depends on the reliability and simplicity of gel filtration as a separation technique. This handbook is a valuable laboratory aid to the selection and use of GE Healthcare gel filtration media.....**23578**

Products for Gel Filtration Chromatography

Description	Sample MW (kDa, globular proteins)	Pore Size (Å)	Particle Size (µm)	I.D. (mm)	Length (cm)	Cat. No.
TSK-GEL® SW Gel Filtration Columns						
Super SW2000 30 cm × 4.6 mm I.D., 4 µm particle size	5-150	125	4	4.6	30	818674
G2000SW _{XL} 30 cm × 7.8 mm I.D., 5 µm particle size	5-150	125	5	7.8	30	808540
G2000SW 30 cm × 7.5 mm I.D., 10 µm particle size	5-100	125	10	7.5	30	805788
G2000SW 60 cm × 7.5 mm I.D., 10 µm particle size	5-100	125	10	7.5	60	805102
Super SW3000 30 cm × 4.6 mm I.D., 4 µm particle size	10-500	250	4	4.6	30	818675
G3000SW _{XL} 30 cm × 7.8 mm I.D., 5 µm particle size	10-500	250	5	7.8	30	808541
G3000SW 30 cm × 7.5 mm I.D., 10 µm particle size	10-500	250	10	7.5	30	805789
G3000SW 60 cm × 7.5 mm I.D., 10 µm particle size	10-500	250	10	7.5	60	805103
G3000SW 60 cm × 21.5 mm I.D., 13 µm particle size	10-500	250	13	21.5	60	805147
G4000SW _{XL} 30 cm × 7.8 mm I.D., 8 µm particle size	20-10,000	450	8	7.8	30	808542
G4000SW 30 cm × 7.5 mm I.D., 13 µm particle size	20-10,000	450	13	7.5	30	805790
G4000SW 60 cm × 7.5 mm I.D., 13 µm particle size	20-10,000	450	13	7.5	60	805104

Products for Gel Filtration Chromatography (Cont.)

Description	Cat. No.
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Gel Filtration Media

Agarose

Agarose, BioChemika, for chromatography, 6 B, beads (6% in water)	05078
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Cellulose

Cellulose for column chromatography, acid washed, powder	22184
Cellulose for column chromatography, powder	22183
Cellulose from spruce, for column chromatography, acid washed	22182

Dextran

Dextran crosslinked G-25, BioChemika	01468
Dextrangel N 25, BioChemika, for column chromatography	31399

Sephacryl®

Sephacryl 100-HR, pore size 1-100 kDa MW range (globular proteins)	S100HR
Sephacryl 200-HR, pore size 5-250 kDa MW range (globular proteins), pore size 1-80 kDa MW range (dextrans)	S200HR
Sephacryl 300-HR, pore size 10-1500 kDa MW range (globular proteins), pore size 1-400 kDa MW range (dextrans)	S300HR
Sephacryl 400-HR, pore size 20-8000 kDa MW range (globular proteins)	S400HR
Sephacryl 500-HR, pore size 40-20,000 kDa MW range (dextrans)	S500HR

Description	Cat. No.
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Sepharose®

Sepharose 2B 60-200 µm bead diameter	2B300
Sepharose 4B 40-165 µm bead diameter	4B200
Sepharose 6B 45-165 µm (wet), pore size 10-1,000 kDa fractionation range (dextrans)	6B100
Sepharose CL-2B Crosslinked	CL2B300
Sepharose CL-4B Crosslinked	CL4B200
Sepharose CL-6B Crosslinked	CL6B200

Superdex®

Superdex 30 Prep Grade	S2792
Superdex 75 Prep Grade	S6657
Superdex 200 Prep Grade	S6782

Sephadex®

Sephadex G-10 Medium	G10120
Sephadex G-15 Medium	G15120
Sephadex G-25 Coarse	G25300
Sephadex G-25 Medium	G25150
Sephadex G-25 Superfine	G2580
Sephadex G-50 Coarse	G50300
Sephadex G-50 Medium	G50150
Sephadex G-50 Fine	G5080
Sephadex G-50 Superfine	G5050
Sephadex G-75 Medium	G75120
Sephadex G-75 Superfine	G7550
Sephadex G-100 Medium	G100120
Sephadex G-100 Superfine	G10050

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