[GPC CAPABILITIES]

Gel Permeation Chromatography (GPC)

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

Gel Permeation Chromatography

Table of Contents

Organic Soluble Polymers

Standards for Non-Aqueous Calibration....4
GPC Columns for Non-Aqueous Samples....6

Water Soluble Polymers and Small Molecules

Standards for Aqueous Calibration....14
SEC Columns for Aqueous Samples....16
SEC Columns for Protein Analysis and Characterization....18

Autosampler Vials

LC/GC Certified Vials....20
TruView LCMS Certified Vials....20
Choosing the Right Vial and Septum for Your Application....21
Vial Closures Guide....21

Tips and Frequently Asked Questions

Solvent Considerations....24
Frequently Asked Questions....26

In 1964 John C. Moore, of the Dow Chemical Company, published his work on the preparation of Gel Permeation Chromatography (GPC) and changed how scientists studied synthetic polymers and macromolecules. Shortly thereafter Waters Corporation licensed the technology from Dow to produce the first commercially-available gel permeation chromatograph, the GPC-100. With dedicated, purpose-built instrumentation combined with the innovations from the Dow Chemical Company it was possible for GPC to provide critical information to scientists that was difficult to obtain by other methods.

For over 40 years, Waters has continued to refine the instrumentation, packing materials, and technology to improve GPC and SEC analysis. These innovations allow size-exclusion techniques to expand outside of the original polymer analysis to include applications for separating small and large molecules from interfering matrices, such as those found in foods, pharmaceutical preparations, and natural products.

As a market leader in GPC analysis, Waters provides you with the highest quality GPC products and expert applications support. As a primary manufacturer of chromatographic instrumentation and consumables, all our facilities follow strict ISO, FDA and cGMP guidelines. This is your assurance that Waters will continue to provide you with solutions that will be at the forefront of separation science.

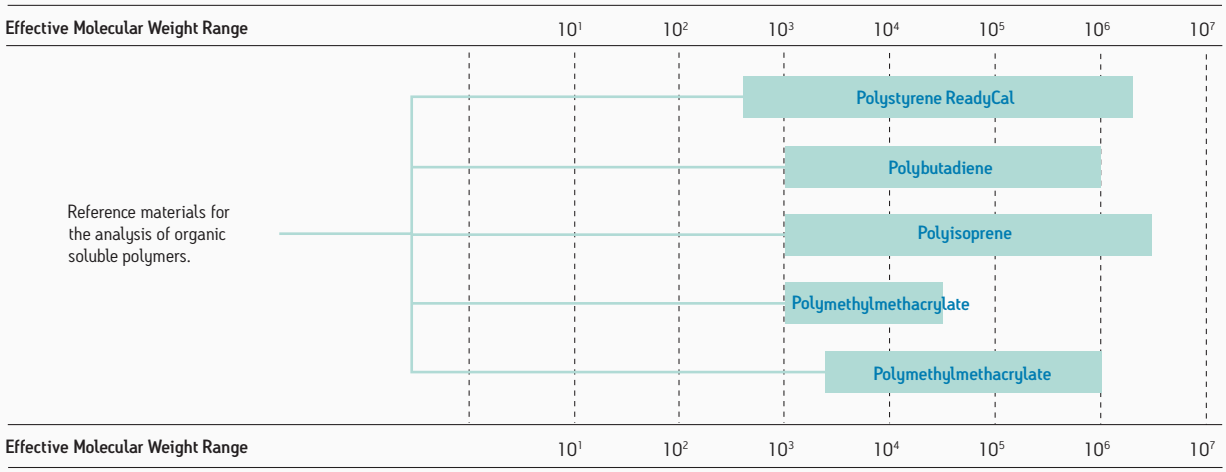
(GPC)

Standards for Non-Aqueous Calibration

Reference Materials for Non-Aqueous Samples

We understand that accurate and reliable data is only achieved using a properly calibrated system. By providing you with well-characterized polymer standards and reference materials we help you to focus on results and maintain your productivity. The polymers used in our reference materials have been specifically manufactured to provide known molecular weight data for a wide range of analysis. Whether your choice is for an individual standard or a cocktail mix, you can count on the traceability of our performance-based reference materials.

Non-Aqueous GPC Standards Guide



ReadyCal Standards

A ReadyCal kit allows you to quickly and accurately prepare a multi-point calibration curve without the need to weigh chemicals. Each vial contains a polymer mix that spans a molecular weight range to provide baseline resolution of each component. Simply add solvent directly to the vial and mix.

Description*	Part No.
Polystyrene ReadyCal Standards 4 mL Kit	WAT058930
A complete kit of ready-to-use polystyrene calibration standards. Kit contains thirty 4 mL autosampler vials which contain four polystyrene standards per vial. There are three separate molecular weight ranges in each kit, ten units of each range. Range is from 400 to 2,000,000 Da	
Polystyrene ReadyCal Standards 2 mL Kit	WAT058931
A complete kit of ready-to-use polystyrene calibration standards. Kit contains thirty 2 mL autosampler vials which contain four polystyrene standards per vial. There are three separate molecular weight ranges in each kit, ten units of each range. Range is from 400 to 2,000,000 Da	

**Values listed are approximate molecular weights.*

Polymer Specific Calibration Standards

Tailored specifically for different types of polymer analysis, these conveniently prepared calibration standards provide the analyst a quick and reliable reference to known molecular weight ranges. Polymer type and MW ranges are specified in the product guide below.

Description*	Part No.
Polybutadiene Standards Kit 0.5 g/vial polybutadiene at each molecular weight: 1000, 3000, 7000, 10,000, 30,000, 70,000, 100,000, 300,000, 700,000, 1,000,000	WAT035709
Polyisoprene Standards Kit 0.5 g/vial polyisoprene at each molecular weight: 1000, 3000, 10,000, 30,000, 70,000, 100,000, 300,000, 500,000, 1,000,000, 3,000,000	WAT035708
Polymethylmethacrylate Low MW Standards Kit 0.5 g/vial polymethylmethacrylate at each molecular weight: 1000, 1700, 2500, 3500, 5000, 7000, 10,000, 13,000, 20,000, 30,000	WAT035707
Polymethylmethacrylate Mid MW Standards Kit 0.5 g/vial polymethylmethacrylate at each molecular weight: 2400, 9500, 31,000, 52,000, 100,000, 170,000, 270,000, 490,000, 730,000, 1,000,000	WAT035706

**Values listed are approximate molecular weights.*

Individual MW Reference Materials

In many cases a single calibration standard is used to verify a molecular weight component in a sample mixture or extend the range of an existing calibration solution. These individual component standards make molecular weight identification simple and straightforward.

Description*	Part No.
Polystyrene Standard 400 10 g/vial polystyrene, 400 MW	WAT011590
Polystyrene Standard 530 10 g/vial polystyrene, 530 MW	WAT011592
Polystyrene Standard 950 10 g/vial polystyrene, 950 MW	WAT011594
Polystyrene Standard 2,800 5 g/vial polystyrene, 2,800 MW	WAT011596
Polystyrene Standard 6,400 5 g/vial polystyrene, 6,400 MW	WAT011598
Polystyrene Standard 10,100 5 g/vial polystyrene, 10,100 MW	WAT011600
Polystyrene Standard 17,000 5 g/vial polystyrene, 17,000 MW	WAT011602
Polystyrene Standard 43,000 5 g/vial polystyrene, 43,000 MW	WAT011604
Polystyrene Standard 110,000 5 g/vial polystyrene, 110,000 MW	WAT011606
Polystyrene Standard 180,000 5 g/vial polystyrene, 180,000 MW	WAT011608

Description*	Part No.
Polystyrene Standard 430,000 5 g/vial polystyrene, 430,000 MW	WAT011612
Polystyrene Standard 780,000 5 g/vial polystyrene, 780,000 MW	WAT011614
Polystyrene Standard 1,300,000 1 g/vial polystyrene, 1,300,000 MW	WAT011616
Polystyrene Standard 2,800,000 1 g/vial polystyrene, 2,800,000 MW	WAT011618
Polystyrene Standard 3,600,000 1 g/vial polystyrene, 3,600,000 MW	WAT011620
Polystyrene Standard 4,300,000 1 g/vial polystyrene, 4,300,000 MW	WAT011622
Polystyrene Standard 5,200,000 1 g/vial polystyrene, 5,200,000 MW	WAT011624
Polystyrene Standard 6,200,000 1 g/vial polystyrene, 6,200,000 MW	WAT011626
Polystyrene Standard 8,400,000 1 g/vial polystyrene, 8,400,000 MW	WAT011628
Polystyrene Standard 20,000,000 1 g/vial polystyrene, 20,000,000 MW	WAT011630

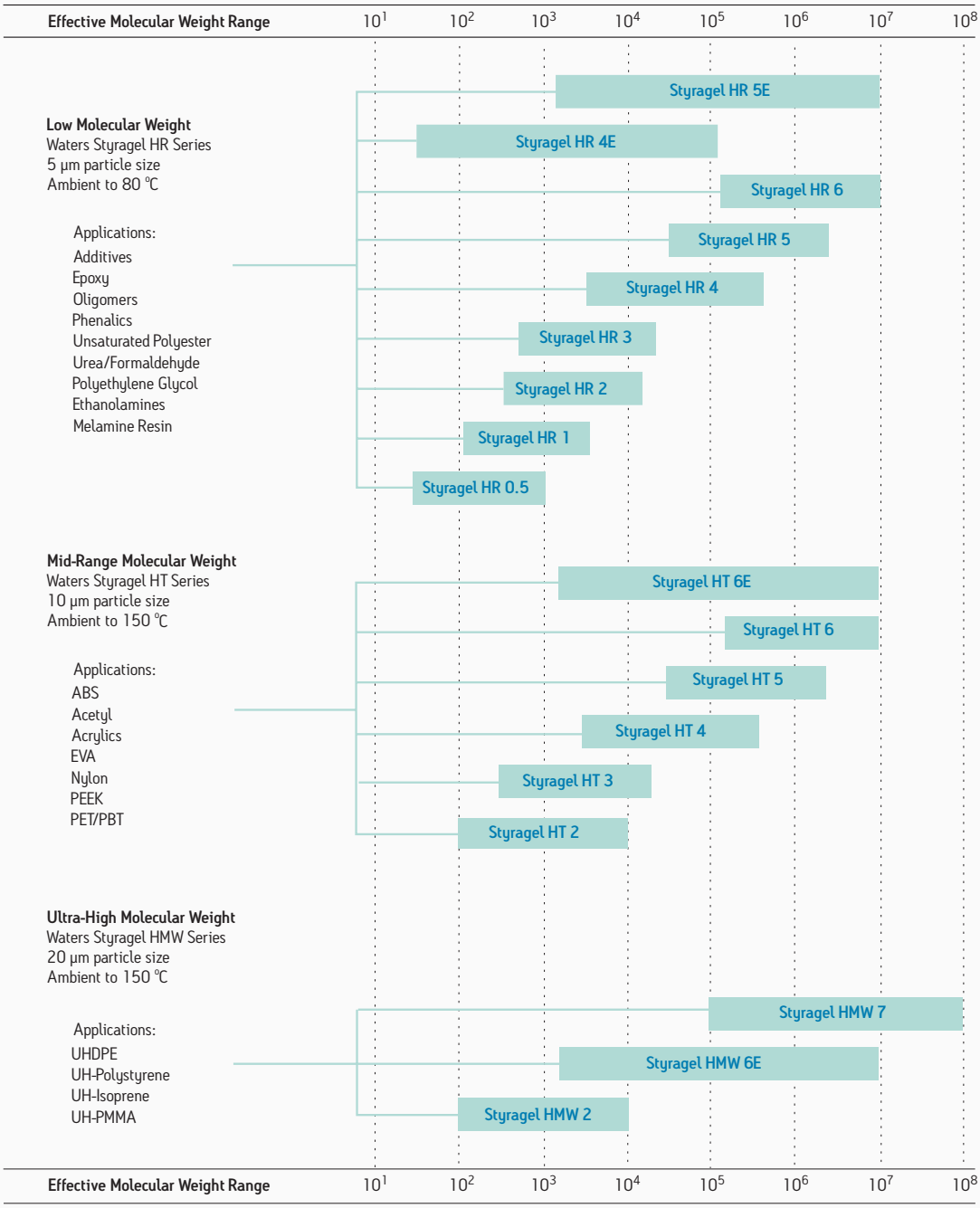
**Values listed are approximate molecular weights.*

GPC Columns for Non-Aqueous Samples

A GPC column is selected based on the goals of the separation, which often ranges from one of maximum speed for screening to that of maximum resolution for determining product quality control. Each analysis provides unique challenges for separation. By providing you with a comprehensive selection of GPC columns, you can be certain that the column or column bank that you choose will be compatible with temperature, solvent, and polymer type.

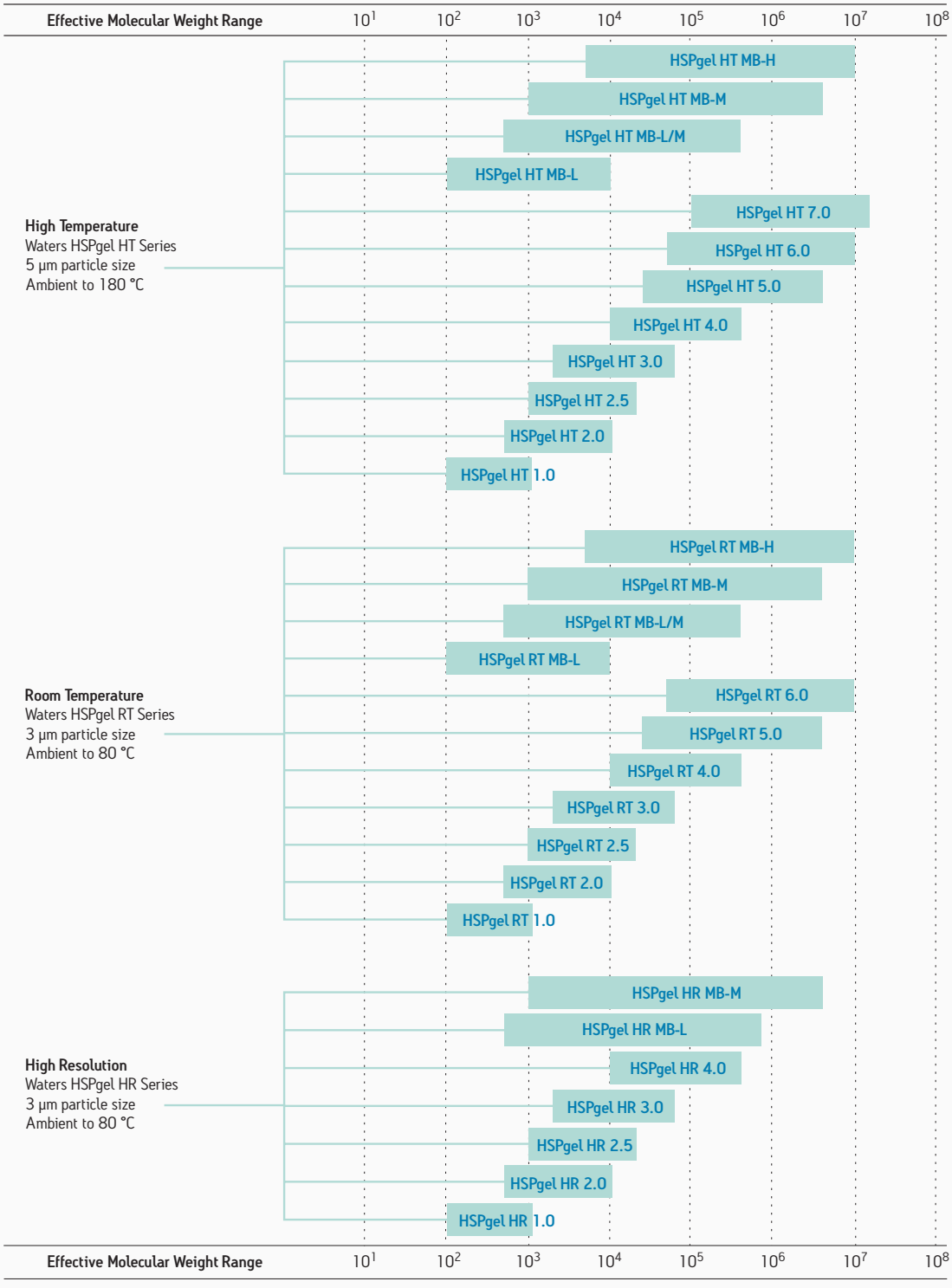
The following charts may be used to quickly compare the molecular weight ranges for the specified columns. By connecting two or more columns in series, the effective molecular weight range can be extended to provide coverage for more complex sample analysis.

Styragel Columns Selection Guide



HMW—High Molecular Weight HT—High Temperature HR—High Resolution

HSPgel Columns Selection Guide*



**MW ranges for HR and RT are based on polystyrene chain lengths.*

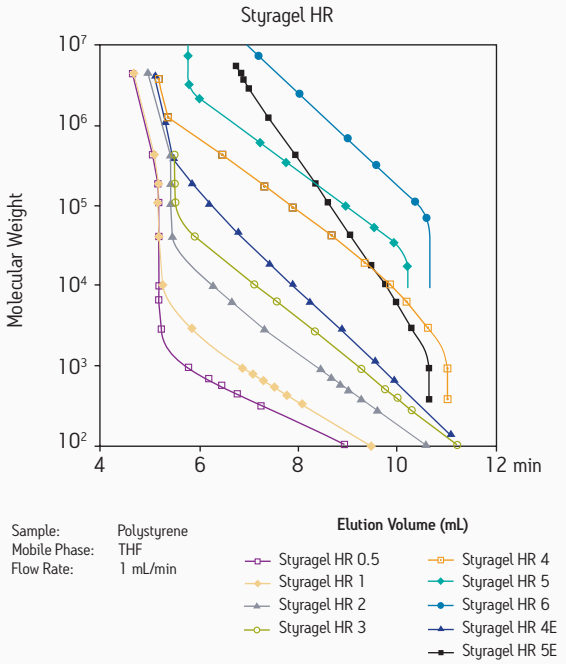
HR—High Resolution
RT—Room Temperature
HT—High Temperature
MB—Mixed Bed
L—Low MW Range
M—Medium MW Range
L/M—Low/Medium MW Range
H—High MW Range

Styragel Columns for Polymer Characterization

Styragel HR (High-Resolution) Columns

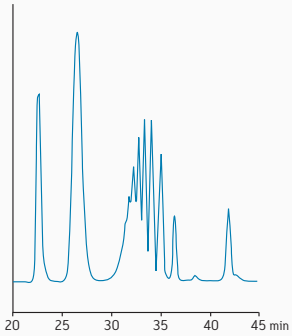
Designed particularly for low molecular weight samples, the Waters Styragel® HR Columns are ideal for the analysis of oligomers, epoxies, and polymer additives where high resolution is critical. Packed with rigid 5-µm particles, these columns deliver unrivaled resolution and efficiency in the low-to-mid molecular weight region.

Calibration Curves for the Waters Styragel HR Series of High-Resolution Columns



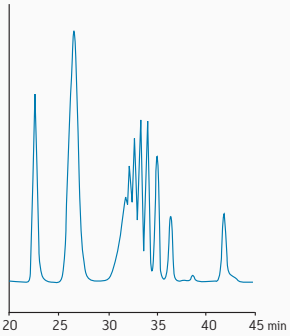
Styragel HR Columns for Unrivaled Resolution of Low Molecular Weight Samples

Column Bank: Styragel HR 0.5, 1, 2, and 3
Part Numbers: WAT044231, WAT044234, WAT044237, WAT044222
Mobile Phase: THF
Column Temp.: 35 °C
Flow Rate: 1 mL/min
Sample: Polystyrene standards: 0.5K, 5.05K, 49.8K



Conventional Styragel HR Columns 7.8 x 300 mm

Column Bank: Styragel HR 0.5, 1, 2, and 3
Part Numbers: WAT045835, WAT045850, WAT045865, WAT045880
Mobile Phase: THF
Column Temp.: 35 °C
Flow Rate: 0.35 mL/min
Sample: Polystyrene standard mix 0.5K, 5.05K, 49.8K



Solvent-Efficient Styragel HR Columns 4.6 x 300 mm

Styragel HR Columns (7.8 x 300 mm)

Column	Effective MW Range	Part No. THF	Part No. DMF	Part No. Toluene
Styragel HR 0.5	0–1,000	WAT044231	WAT044232	WAT044230
Styragel HR 1	100–5,000	WAT044234	WAT044235	WAT044233
Styragel HR 2	500–20,000	WAT044237	WAT044238	WAT044236
Styragel HR 3	500–30,000	WAT044222	WAT044223	WAT044221
Styragel HR 4	5,000–600,000	WAT044225	WAT044226	WAT044224
Styragel HR 4E	50–100,000	WAT044240	WAT044241	WAT044239
Styragel HR 5	50,000–4,000,000	WAT054460	WAT054466	WAT054464
Styragel HR 5E	2,000–4,000,000	WAT044228	WAT044229	WAT044227
Styragel HR 6	200,000–10,000,000	WAT054468	WAT054474	WAT054470
Styragel Guard Column 4.6 x 30 mm	—	WAT054405	WAT054415	WAT054410

Styragel HR Columns (4.6 x 300 mm)

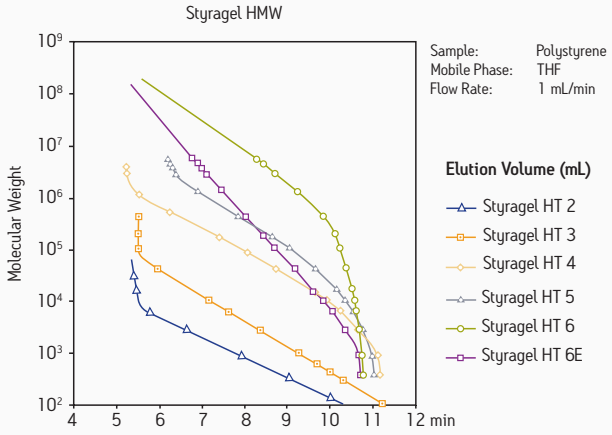
The 4.6 x 300 mm solvent-efficient Styragel Columns offer the same high resolution performance as our conventional 7.8 x 300 mm Styragel Columns, with the added advantage of reducing solvent consumption by two-thirds.

Column	Effective MW Range	Part No. THF	Part No. DMF	Part No. Toluene
Styragel HR 0.5	0–1,000	WAT045835	WAT045840	WAT045830
Styragel HR 1	100–5,000	WAT045850	WAT045855	WAT045845
Styragel HR 2	500–20,000	WAT045865	WAT045870	WAT045860
Styragel HR 3	500–30,000	WAT045880	WAT045885	WAT045875
Styragel HR 4	5,000–600,000	WAT045895	WAT045900	WAT045890
Styragel HR 4E	50–100,000	WAT045805	WAT045810	WAT045800
Styragel HR 5E	2,000–4,000,000	WAT045820	WAT045825	WAT045815

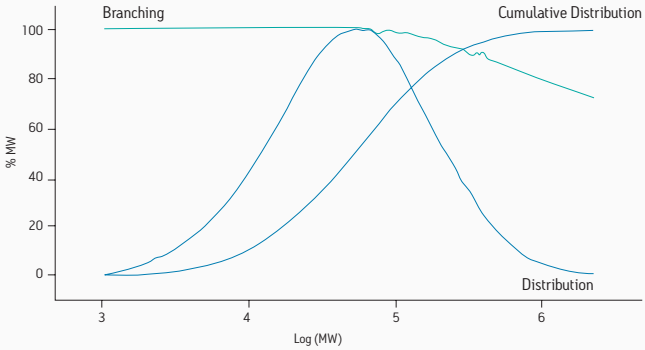
Styragel HT (High-Temperature) Columns

The Styragel HT Columns can be used with aggressive solvents at high temperatures without sacrificing resolution or column lifetime. Packed with rigid 10-µm particles, they have a typical plate count greater than 10,000 plates per column. These columns are extremely durable due to a narrow particle size distribution that results in a very stable column bed. Suitable for both ambient and high-temperature analysis, the Styragel HT Columns offer excellent resolution of polymers in the mid-to-high molecular weight range.

Calibration Curves for the Waters Styragel HT Series of High-Temperature Columns



Styragel HT Columns Deliver Superior Performance — Even at High Temperatures



Column: Styragel HT 6E
Part Number: WAT044218
Mobile Phase: TCB
Column Temp.: 140 °C
Sample: LDPE

Styragel HT Columns (7.8 x 300 mm)

Column	Effective MW Range	Part No. THF	Part No. DMF	Part No. Toluene
Styragel HT 2	100–10,000	WAT054475	WAT054480	WAT054476
Styragel HT 3	500–30,000	WAT044207	WAT044208	WAT044206
Styragel HT 4	5,000–600,000	WAT044210	WAT044211	WAT044209
Styragel HT 5	50,000–4,000,000	WAT044213	WAT044214	WAT044212
Styragel HT 6	200,000–10,000,000	WAT044216	WAT044217	WAT044215
Styragel HT 6E	5,000–10,000,000	WAT044219	WAT044220	WAT044218
Styragel Guard Column 4.6 x 30 mm	—	WAT054405	WAT054415	WAT054410

Styragel HT Columns (4.6 x 300 mm)

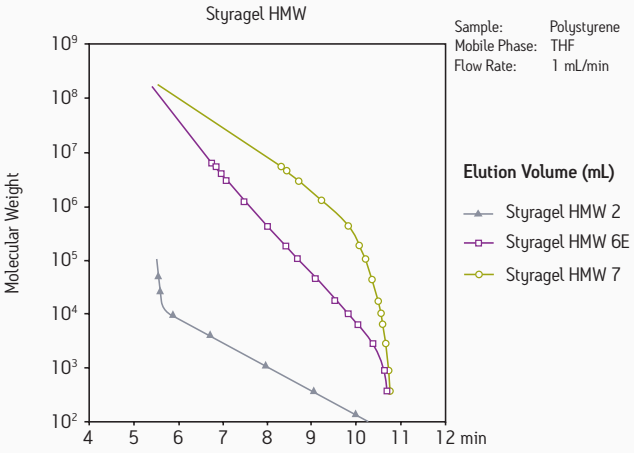
The same high performance as our conventional Styragel HT Columns with the added advantage of reducing your solvent consumption by two-thirds.

Column	Effective MW Range	Part No. THF	Part No. DMF	Part No. Toluene
Styragel HT 3	500–30,000	WAT045920	WAT045925	WAT045915
Styragel HT 4	5,000–600,000	WAT045935	WAT045940	WAT045930
Styragel HT 5	50,000–4,000,000	WAT045950	WAT045955	WAT045945
Styragel HT 6	200,000–10,000,000	WAT045965	WAT045970	WAT045960
Styragel HT 6E	5,000–10,000,000	WAT045980	WAT045985	WAT045975

Styragel HMW (High-Molecular Weight) Columns

The Styragel HMW Columns were specifically designed for the analysis of ultra-high molecular weight polymers susceptible to shearing. Combining high-porosity 10-µm frits and 20-µm particles, the Styragel HMW Columns minimize polymer shear effects. These state-of-the-art columns can be used at ambient or elevated temperatures, and exhibit excellent column lifetime.

Calibration Curves for Waters Styragel HMW Series of High-Molecular Weight Columns



Styragel HMW Columns (7.8 x 300 mm)

Column	Effective MW Range	Part No. THF	Part No. DMF	Part No. Toluene
Styragel HMW 2	100–10,000	WAT054488	WAT054494	WAT054490
Styragel HMW 7	500,000–1 x 10^8	WAT044201	WAT044202	WAT044200
Styragel HMW 6E	5,000–1 x 10^7	WAT044204	WAT044205	WAT044203
Styragel Guard Column 4.6 x 30 mm	—	WAT054405	WAT054415	WAT054410

Styragel HMW Columns (4.6 x 300 mm)

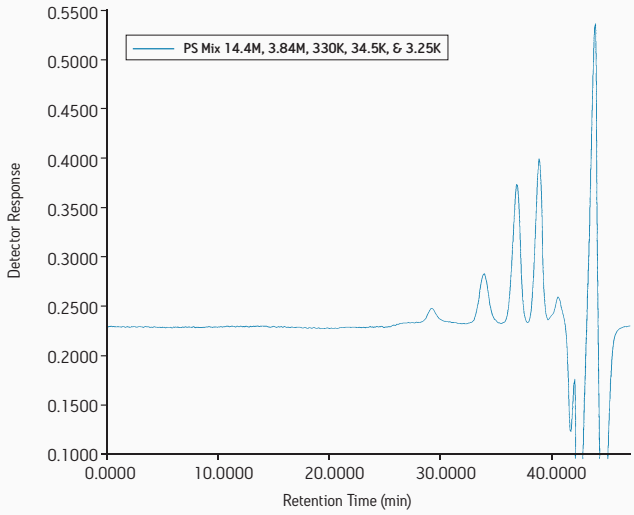
The same high performance as our conventional Styragel HMW Columns with the added advantage of reducing your solvent consumption by two-thirds.

Column	Effective MW Range	Part No. THF	Part No. DMF	Part No. Toluene
Styragel HMW 7	500,000–1 x 10^8	WAT046805	WAT046810	WAT046800
Styragel HMW 6E	5,000–1 x 10^7	WAT046820	WAT046825	WAT046815

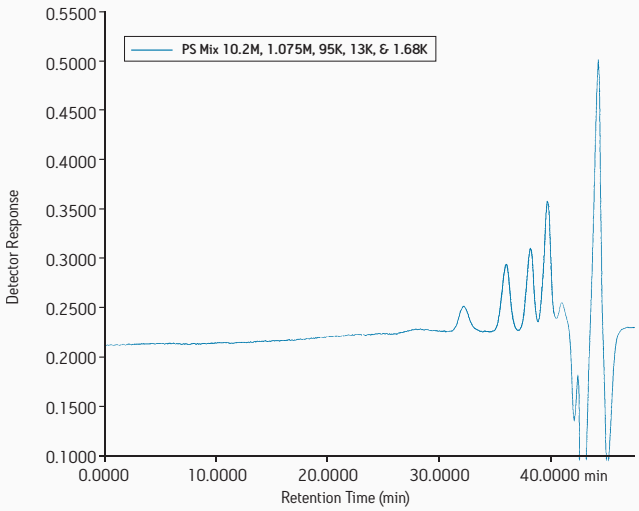
**System dead volume must be minimized for maximum column performance.*

Styragel HMW Columns are Optimized for Analysis of Shear-Sensitive, Ultra-High Molecular Weight Polymers

Column Bank: 2 Styragel HMW 7 and 2 Styragel HMW 6E
Part Numbers: WAT044200 & WAT044203
Column Dimensions: 7.8 x 300 mm
Polystyrene Stds: 14.4M, 3.84M, 330K, 34.5K, 3.25K
Flow Rate: 1 mL/min
Column Temp.: 145 °C
Solvent: TCB



Column Bank: 2 Styragel HMW 7 and 2 Styragel HMW 6E
Part Numbers: WAT044200 & WAT044203
Column Dimensions: 7.8 x 300 mm
Polystyrene Stds: 10.2M, 1.075M, 95K, 13K, 1.68K
Flow Rate: 1 mL/min
Column Temp.: 145 °C
Solvent: TCB



Ultrastyragel Columns

Ultrastyragel™ Preparative Columns provide high-efficiency GPC separations for compound isolation and sample clean-up. Closely related to Styragel GPC Columns, the family of Ultrastyragel Columns provide a two- to three-fold increase in efficiency (plates/meter) that improves separation speed and reduces solvent consumption for preparative isolation. Separations that once required several smaller Styragel Columns can be performed on a single, more efficient Ultrasytragel Preparative Column.

Ultrastyragel Columns (19 x 300 mm)

For high resolution preparative applications, these columns are available in toluene or THF.

Pore Size	Effective MW Range	Flow Rate (mL/min)	Part No. Toluene	Part No. THF
100 Å	50–1,500	4–10	WAT025866	WAT025859
500 Å	100–10,000	4–10	WAT025867	WAT025860
1000 Å	200–30,000	4–10	WAT025868	WAT025861
10,000 Å	5,000–600,000	4–10	WAT025869	WAT025862
100,000 Å	50,000–4,000,000	4–10	WAT025870	WAT025863
1,000,000 Å	200,000–10,000,000	4–10	WAT025871	WAT025864
Linear	2,000–4,000,000	4–10	WAT025872	WAT025865

HSPgel Columns for High-Speed GPC Analysis

Waters HSPgel™ Column offering for high-speed GPC analysis, provides accurate and precise molecular weight determination, increased sample throughput, and greatly reduced solvent consumption and disposal. Waters offers a series of 6.0 x 150 mm high-speed GPC columns.

- HSPgel HR series for high resolution, room temperature GPC
- HSPgel RT series for routine room temperature GPC
- HSPgel HT series for high temperature GPC

The **HSPgel HR series** is designed for high resolution, room temperature, organic polymer GPC. These columns are packed in THF and can be converted once to toluene, methylene chloride, or chloroform.

Column	Solvent	Particle Size	MW Range	Part No.
Ultra-High Resolution GPC*				
HSPgel HR 1.0	THF	3 µm	100–1,000	186001741
HSPgel HR 2.0	THF	3 µm	500–10,000	186001742
HSPgel HR 2.5	THF	3 µm	1,000–20,000	186001743
HSPgel HR 3.0	THF	3 µm	2,000–60,000	186001744
HSPgel HR 4.0	THF	3 µm	10,000–400,000	186001745
HSPgel HR MB-L	THF	3 µm	500–700,000	186001746
HSPgel HR MB-M	THF	3, 5 µm	1,000–4,000,000	186001747

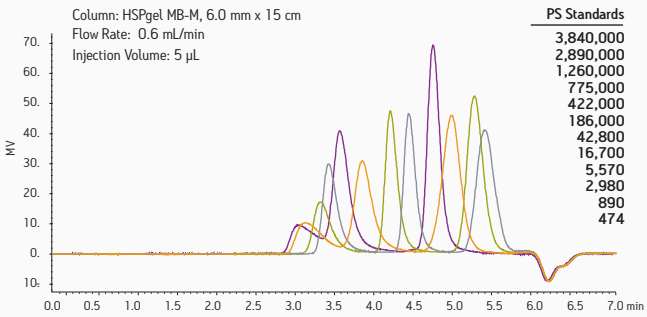
The **HSPgel RT series** are designed for room temperature, routine work of organic polymer GPC. These come packed in THF and can be converted multiple times from THF to toluene, chloroform, methylene chloride, DMF, DMSO, etc.

Column	Solvent	Particle Size	MW Range	Part No.
Room-Temperature GPC*				
HSPgel RT 1.0	THF	3 µm	100–1,000	186001749
HSPgel RT 2.0	THF	3 µm	500–10,000	186001750
HSPgel RT 2.5	THF	3 µm	1,000–20,000	186001751
HSPgel RT 3.0	THF	3 µm	2,000–60,000	186001752
HSPgel RT 4.0	THF	3 µm	10,000–400,000	186001753
HSPgel RT 5.0	THF	3 µm	25,000–4,000,000	186001754
HSPgel RT 6.0	THF	5 µm	50,000–10,000,000	186001755
HSPgel RT MB-L	THF	3 µm	100–10,000	186001757
HSPgel RT MB-L/M	THF	3 µm	500–400,000	186001758
HSPgel RT MB-M	THF	3 µm	1,000–4,000,000	186001759
HSPgel RT MB-H	THF	3, 5 µm	5,000–10,000,000	186001760

**MW ranges for HR and RT are based on polystyrene chain lengths*

HR—High Resolution
RT—Room Temperature
HT—High Temperature
MB—Mixed Bed
L—Low MW Range
M—Medium MW Range
L/M—Low/Medium MW Range
H—High MW Range

High Speed GPC of Polystyrene Standards



The **HSPgel HT series** are designed for room temperature to high temperature (180 °C) organic GPC. The columns come shipped in either THF or ODCB. The ODCB packed column should be used for direct conversion to TCB. These columns can withstand multiple solvent switches.

Column	Solvent	Particle Size	MW Range	Part No.
High-Temperature GPC				
HSPgel HT 1.0	THF	5 µm	100–1,000	186001761
HSPgel HT 2.0	THF	5 µm	500–10,000	186001762
HSPgel HT 2.5	THF	5 µm	1,000–20,000	186001763
HSPgel HT 3.0	THF	5 µm	2,000–60,000	186001764
HSPgel HT 4.0	THF	5 µm	10,000–400,000	186001765
HSPgel HT 5.0	THF	5 µm	25,000–4,000,000	186001766
HSPgel HT 6.0	THF	5 µm	50,000–10,000,000	186001767
HSPgel HT 7.0	THF	5 µm	100,000–15,000,000	186001768
HSPgel HT MB-L	THF	5 µm	100–1,000	186001769
HSPgel HT MB-L/M	THF	5 µm	500–400,000	186001770
HSPgel HT MB-M	THF	5 µm	1,000–4,000,000	186001771
HSPgel HT MB-H	THF	5 µm	5,000–10,000,000	186001772
HSPgel HT 1.0	ODCB	5 µm	100–1,000	186001773
HSPgel HT 2.0	ODCB	5 µm	500–10,000	186001774
HSPgel HT 2.5	ODCB	5 µm	1,000–20,000	186001775
HSPgel HT 3.0	ODCB	5 µm	2,000–60,000	186001776
HSPgel HT 4.0	ODCB	5 µm	10,000–400,000	186001777
HSPgel HT 5.0	ODCB	5 µm	25,000–4,000,000	186001778
HSPgel HT 6.0	ODCB	5 µm	50,000–10,000,000	186001779
HSPgel HT 7.0	ODCB	5 µm	100,000–15,000,000	186001780
HSPgel HT MB-L	ODCB	5 µm	100–1,000	186001781
HSPgel HT MB-L/M	ODCB	5 µm	500–400,000	186001782
HSPgel HT MB-M	ODCB	5 µm	1,000–4,000,000	186001783
HSPgel HT MB-H	ODCB	5 µm	5,000–10,000,000	186001784

Shodex GPC Columns

Waters is proud to distribute Shodex™ GPC Columns and accessories. For over 25 years, Shodex GPC Columns have been used successfully by scientists worldwide. The following selection of highly-reproducible GPC columns contains styrene divinylbenzene resins.

K-800 Series (8 x 300 mm)

Ultra-high-efficiency columns designed for high-resolution performance. They are available in THF, DMF, or chloroform.

Type	Polystyrene Exclusion Limit	Part No.
KF-800 (THF)		
KF-801	1500	WAT030697
KF-802	5000	WAT030698
KF-802.5	20,000	WAT030699
KF-803	70,000	WAT034100
KF-804	400,000	WAT034101
KF-805	4,000,000	WAT034102
KF-807	200,000,000	WAT034104
KF-806M (linear)	40,000,000	WAT034105
KF-G Pre-column (4.6 x 10 mm)	—	WAT034106
K-800 (Chloroform)		
K-802.5	20,000	WAT034109
K-803	70,000	WAT034110
K-804	400,000	WAT034111
K-805	4,000,000	WAT034112
K-G Pre-column (4.6 x 10 mm)	—	WAT035524
KD-800 (DMF)		
KD-801	2500	WAT034116
KD-802	5000	WAT034117
KD-802.5	20,000	WAT034118
KD-803	70,000	WAT034119
KD-804	400,000	WAT034120
KD-806	40,000,000	WAT034122
KD-807	200,000,000	WAT034123
KD-806M (linear)	40,000,000	WAT034124
KD-G Pre-column (4.6 x 10 mm)	—	WAT034125
KS-800		
KS-801	1000	WAT034276
KS-802	10,000	WAT034277
KS-804	400,000	WAT034279
KS-800 Pre-column (4.6 x 10 mm)	—	WAT034282

HFIP-800 Series (8 x 300 mm)

These columns have the same high efficiency as the K-series columns but are available in HFIP.

Type	Polystyrene Exclusion Limit	Part No.
HFIP-803	70,000	WAT035605
HFIP-806M (linear)	40,000,000	WAT035611
HFP-LG Pre-column (8 x 50 mm)	—	WAT035612

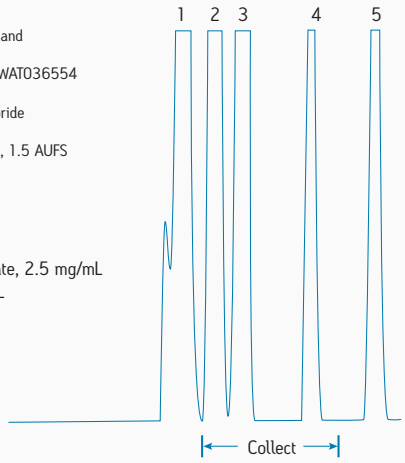
Envirogel High-Resolution GPC Cleanup Columns

The Envirogel™ High-Efficiency GPC Cleanup Columns are specifically designed to remove low volatility, high-molecular-weight interferences, such as lipids and natural resins, from environmental samples as specified in EPA Method 3640A. In the past, the cleanup procedure for environmental samples was performed on low-efficiency GPC columns based on packing particle diameters of 37–75 µm (200–400 mesh) Bio-Beads® S-X resins. The high-efficiency Envirogel GPC Cleanup Columns increase the speed of this process while simultaneously reducing solvent consumption.

Column Optimization

Column: Envirogel GPC, 19 x 300 mm and 19 x 150 mm
Part Numbers: WAT036555, WAT036554
Sample: 2000 µL
Solvent: Methylene chloride
Flow Rate: 5 mL/min
Detection: UV @ 254 nm, 1.5 AUFS

Compounds:
1. Corn oil, 62.5 mg/mL
2. Bis(2-Ethylhexyl) Phthalate, 2.5 mg/mL
3. Methoxychlor, 0.5 mg/mL
4. Perylene, 0.05 mg/mL
5. Sulfur, 0.2 mg/mL



For optimum capacity and resolution, a 150 mm column is used in series with the 300 mm column. The use of both the 150 mm column and the 300 mm column provides maximum loading capacity while the 300 mm column provides maximum throughput and reduction in solvent consumption when used alone.

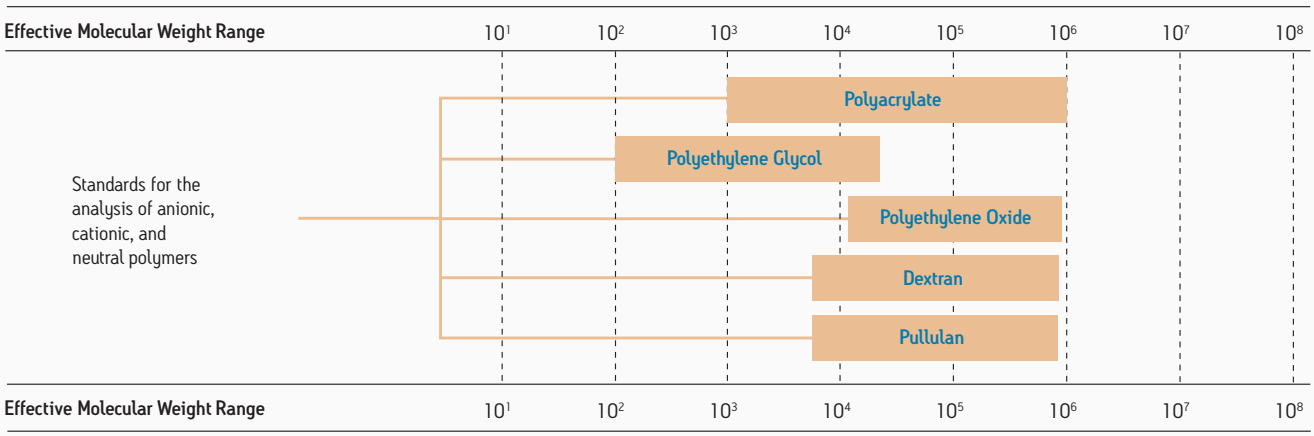
Column	Solvent	Dimension	Part No.
Envirogel GPC Cleanup	Methylene Chloride	19 x 150 mm	WAT036555
Envirogel GPC Cleanup	Cyclohexane/Ethyl Acetate	19 x 150 mm	186001915
Envirogel GPC Cleanup	Methylene Chloride	19 x 300 mm	WAT036554
Envirogel GPC Cleanup	Cyclohexane/Ethyl Acetate	19 x 300 mm	186001916
Envirogel GPC Guard	Methylene Chloride	4.6 x 30 mm	186001913
Envirogel GPC Guard	Cyclohexane/Ethyl Acetate	4.6 x 30 mm	186001914

Standards for Aqueous Calibration

Reference Materials for Aqueous Samples

Reliable SEC results depend on the quality of the reference materials used for the molecular weight calibration. Waters SEC calibration standards are precisely formulated to provide you with accurate molecular weight reference materials that are conveniently packaged to minimize errors in SEC calibration methods. Our fully traceable aqueous-based polymer reference kits simplify routine calibration procedures that improve your workflow and increase your productivity.

Aqueous SEC Standards Guide



This chart may be used to determine the appropriate component standard and corresponding molecular weight range. This information can be used in conjunction with the full range of aqueous SEC standards listed on page 15.

Full-Range Calibration Standards for SEC

These conveniently prepared and prepackaged standards provide you with an accurate calibration range for molecular weight determination of common water soluble polymers. The kits contain a series of well-characterized standards of the specified polymer type and include certificates that list component ranges and concentrations.

Description*	Part No.
Polyacrylic Acid Standards Kit 250 mg/vial polyacrylic acid at each molecular weight: 1000, 3000, 7000, 15,000, 30,000, 70,000, 100,000, 300,000, 700,000 and 1,000,000	WAT035714
Polyethylene Glycol Standards Kit 1.0 g/vial polyethylene glycol at each molecular weight: 100, 200, 400, 600, 1000, 1500, 4300, 7000, 13,000 and 22,000	WAT035711
Polyethylene Oxide Kit 500 mg/vial polyethylene oxide at each molecular weight: 24,000, 40,000, 79,000, 160,000, 340,000, 570,000, and 850,000	WAT011574
Dextrans Standard 500 mg/vial dextrans at each molecular weight: 5000, 12,000, 24,000, 48,000, 148,000, 273,000, 410,000 and 750,000	WAT054392
Pullulan Kit 200 mg/vial pullulan at each molecular weight: 5000, 10,000, 20,000, 50,000, 100,000, 200,000, 400,000, and 800,000	WAT034207

**Values listed are approximate molecular weights.*

Individual Calibration Standards for SEC

In many cases a single calibration standard is used to verify a molecular weight component in a sample mixture. These individual component standards make molecular weight identification simple and straightforward. Package quantity: 0.5 g.

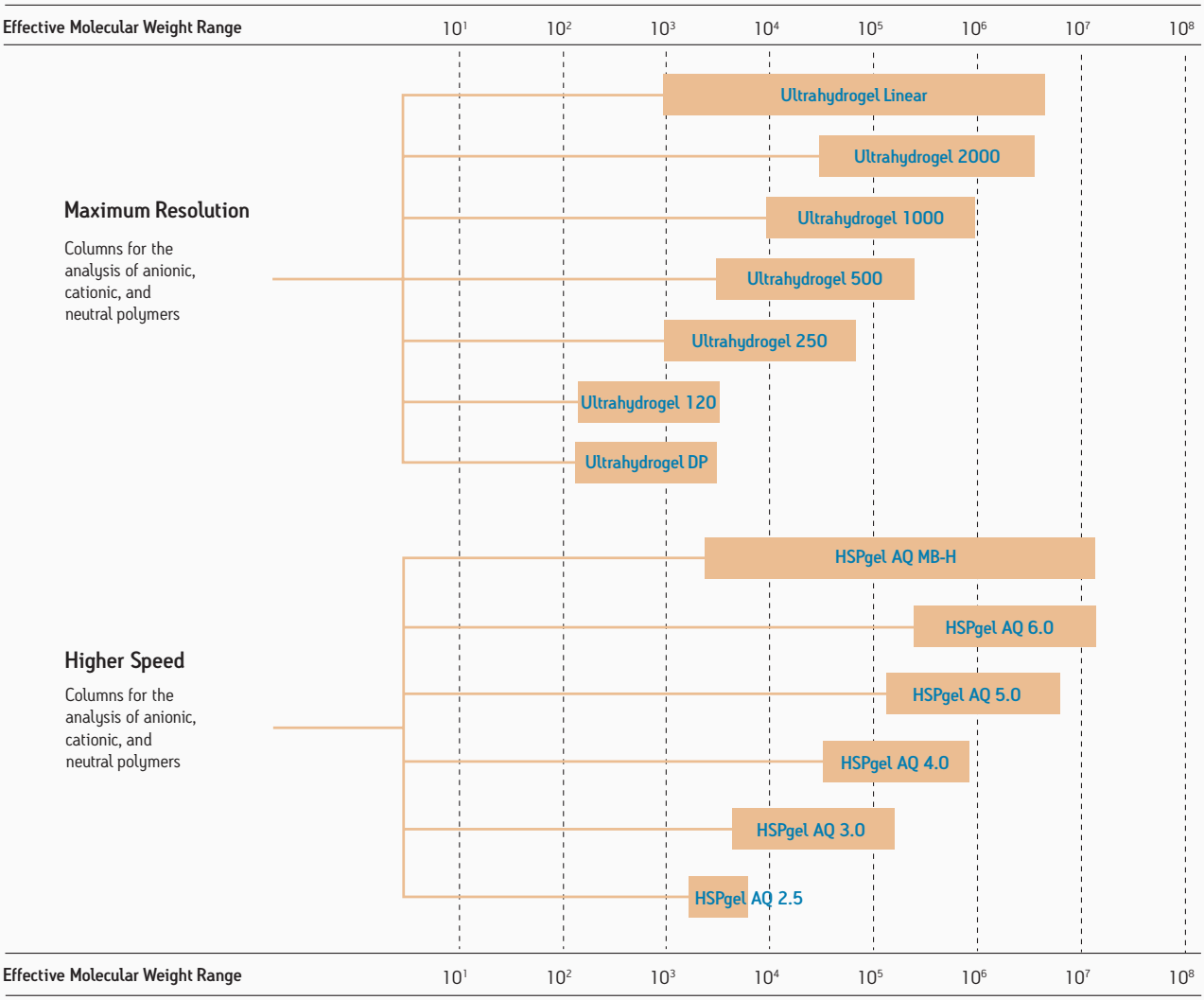
Description*	Part No.
Polyethylene Oxide Standard 24,000 Polyethylene oxide, 24,000 MW	WAT011574
Polyethylene Oxide Standard 40,000 Polyethylene oxide, 40,000 MW	WAT011576
Polyethylene Oxide Standard 79,000 Polyethylene oxide, 79,000 MW	WAT011578
Polyethylene Oxide Standard 160,000 Polyethylene oxide, 160,000 MW	WAT011580
Polyethylene Oxide Standard 340,000 Polyethylene oxide, 340,000 MW	WAT011582
Polyethylene Oxide Standard 570,000 Polyethylene oxide, 570,000 MW	WAT011584
Polyethylene Oxide Standard 850,000 Polyethylene oxide, 850,000 MW	WAT011586

**Values listed are approximate molecular weights.*

SEC Columns for Aqueous Samples

Size Exclusion Chromatography (SEC) and Gel Filtration Chromatography (GFC) are synonymous techniques that are used to separate macromolecules in aqueous environments based on their hydrodynamic volume. Waters SEC columns allow scientists to efficiently separate cationic, anionic and non-ionic macromolecules under a wide range of physical, chemical and biological environments. Whether you are choosing a column bank for maximum molecular weight resolution or selecting a column for quick screening, you can count on the stability, lifetime and performance of a Waters Ultrahydrogel™ SEC Column or HSPgel SEC Column.

Aqueous SEC Column Selection Guide



This chart compares the molecular weight ranges for the specified columns. By connecting two or more columns in series, the effective molecular weight range can be extended to provide coverage for more complex sample analysis.

Ultrahydrogel Columns

Packed with hydroxylated polymethacrylate-based gel, Waters Ultrahydrogel SEC Columns are ideal for the analysis of aqueous-soluble samples, such as oligomers; oligosaccharides; polysaccharides; and cationic, anionic, and amphoteric polymers. Measuring 7.8 x 300 mm, these high-resolution columns offer many advantages over conventional aqueous SEC columns, such as:

- Wide-pH range (2-12)
- Compatibility with high concentrations of organic solvents (up to 20% organic, 50% organic if the mobile phase is introduced by gradient)
- Greater flexibility for the mobile phase
- Minimal non-size-exclusion effects

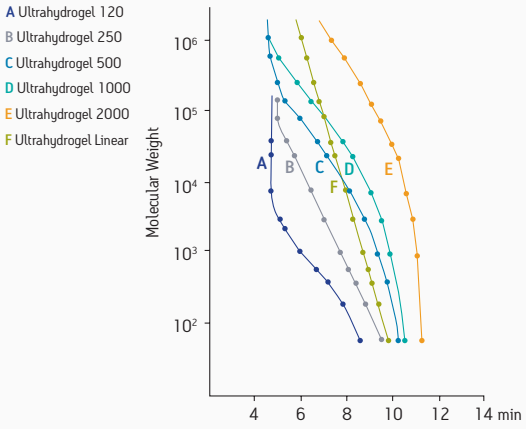
Ultrahydrogel Columns (7.8 x 300 mm)*

Column	Pore Size	Exclusion Limit	Part No.
Ultrahydrogel 120	120 Å	5000	WAT011520
Ultrahydrogel 250	250 Å	80,000	WAT011525
Ultrahydrogel 500	500 Å	400,000	WAT011530
Ultrahydrogel 1000	1000 Å	1,000,000	WAT011535
Ultrahydrogel 2000	2000 Å	7,000,000	WAT011540
Ultrahydrogel Linear	Blend	7,000,000	WAT011545
Ultrahydrogel DP*	120 Å	5000	WAT011550
Ultrahydrogel Guard Column	N/A	N/A	WAT011565
Ultrahydrogel Guard Column DP*	N/A	N/A	WAT011570

**DP = Degree of Polymerization, choice of column when working with glucose oligomers.*

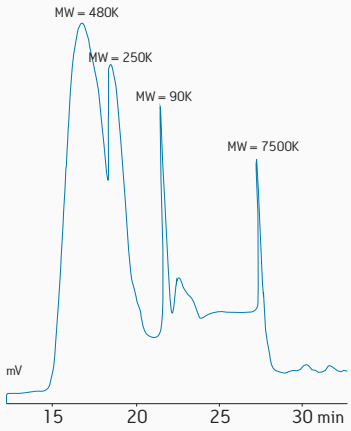
Ultrahydrogel Columns Calibration Curves

Column Dimensions: 7.8 x 300 mm
Part Numbers: WAT011525, WAT011530, WAT011535
Sample: PEG and PEO standards
Mobile Phase: Distilled water
Flow Rate: 1 mL/min



Gelatin Sample

Column: Ultrahydrogel 250, 500, and 1000
Part Numbers: WAT011525, WAT011530, WAT011535
Temperature: 80 °C
Eluent: Water, pH 7, phosphate buffer
Flow Rate: 1 mL/min
Detection: Waters 410 Differential Refractometer



HSPGel Columns

Waters HSPgel SEC Columns are optimized for high-speed polymer analysis in aqueous solution. HSPgel Columns will reduce solvent consumption, increase throughput and provide accurate molecular weight data for any room-temperature analysis. The column dimensions are 6.0 x 150 mm.

HSPgel Columns for High-Speed GPC Analysis

Aqueous GPC**	Solvent	Particle Size	MW Range	Part No.
HSPgel AQ 2.5	Water	4 µm	500–2,000	186001785
HSPgel AQ 3.0	Water	4 µm	1,000–60,000	186001786
HSPgel AQ 4.0	Water	6 µm	10,000–400,000	186001787
HSPgel AQ 5.0	Water	7 µm	50,000–4,000,000	186001788
HSPgel AQ 6.0	Water	9 µm	100,000–10,000,000	186001789
HSPgel AQ MB-H	Water	9 µm	500–10,000,000	186001790

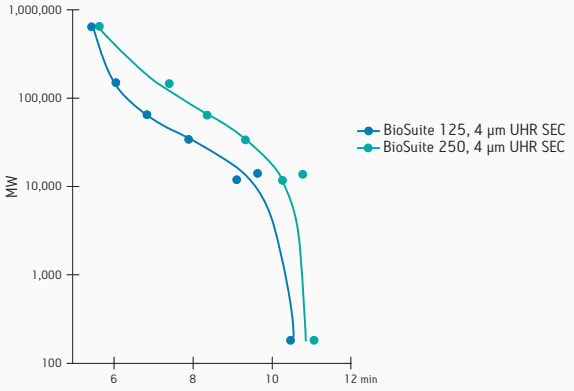
***Exclusion limits for AQ series extrapolated from highest MW PEO standard (~900,000).*

SEC Columns for Protein Analysis and Characterization

BioSuite Size-Exclusion HPLC Columns

BioSuite™ Ultra-High Resolution (UHR), High Resolution (HR), and Standard Size-Exclusion Columns contain silica-based sorbents that are stable from pH 2.5–7.5. As indicated in the calibration curve tables, the exclusion limit of the BioSuite SEC packings is determined by the pore size of the silica-base material. The particle size of the SEC packing media, as well as column length, is an important parameter that determines the separation efficiency. The BioSuite UHR Columns (4-µm particle size) provide maximum separation efficiency, followed by BioSuite HR Columns (5- and 8-µm particle sizes) and BioSuite Standard SEC Columns (10-, 12- and 17-µm particle sizes). To maximize column life of analytical (4.6- or 7.8-mm ID) or preparative (21.5-mm ID) SEC columns, use of BioSuite Guard Columns

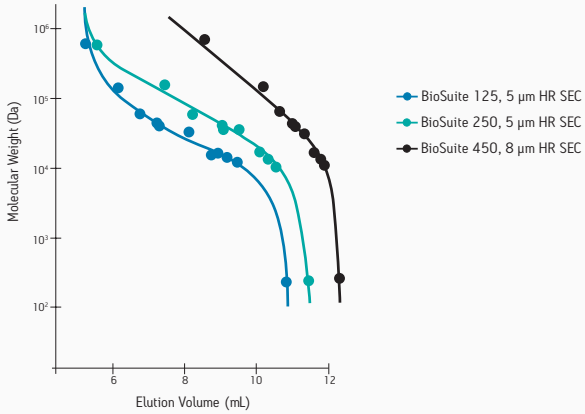
Protein Calibration Curves for BioSuite Ultra-High Resolution (UHR) SEC Columns



Sample: Thyroglobulin (MW 670,000 Da), Gamma globulin (MW 155,000 Da), Boviine serum albumin (66,330 Da), Beta lactoglobulin (MW18,400 Da), Lysozyme (14,300 Da), Cytochrome C (12,400 Da), Triglycine (189 Da)
Columns: BioSuite 250, UHR SEC, 4.6 x 300 mm, 4 µm
BioSuite 125, UHR SEC, 4.6 x 300 mm, 4 µm
Part Numbers: 186002162 (BioSuite 250) & 186002161 (BioSuite 125)
Eluent: 0.15 M sodium phosphate, pH 6.8
Flow Rate: 0.35 mL/min
Column Temp.: 25 °C
Detection: UV @ 220 nm

Column	Globular Protein MW Range	Branched Dextrans	Linear PEG/PEO
BioSuite 125	5,000–150,000	1,000–30,000	500–15,000
BioSuite 250	10,000–500,000	2,000–70,000	1,000–35,000

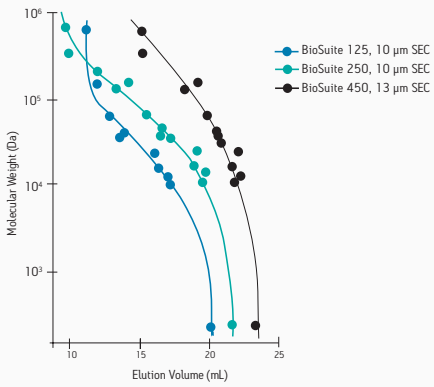
Protein Calibration Curves for BioSuite High Resolution (HR) SEC Columns



Sample: Thyroglobulin (MW 670,000 Da), IgG (MW 156,000 Da), BSA (66,330 Da), Ovalbumin (MW 43,000 Da), Peroxidase (40,200 Da), Beta lactoglobulin (MW 18,400 Da), Myoglobin (MW 16,900 Da), Ribonuclease A (MW 13,700 Da), Cytochrome C (12,400 Da), Glycine tetramer (246 Da)
Columns: BioSuite 450, HR SEC, 7.8 x 300 mm, 8 µm
BioSuite 250, HR SEC, 7.8 x 300 mm, 5 µm
BioSuite 125, HR SEC, 7.8 x 300 mm, 5 µm
Part Numbers: 186002166 (BioSuite 450), 186002165 (Biosuite 250), & 186002164 (BioSuite 125)
Eluent: 0.1 M sodium phosphate, pH 7.0 containing 0.3 M sodium chloride
Flow Rate: 1.0 mL/min
Column Temp.: 25 °C
Detection: UV @ 220 nm

Column	Globular Protein MW Range	Branched Dextrans	Linear PEG/PEO
BioSuite 125	5,000–150,000	1,000–30,000	500–15,000
BioSuite 250	10,000–500,000	2,000–70,000	1,000–35,000

Protein Calibration Curves for BioSuite Standard SEC Columns



Sample: Thyroglobulin (MW 670,000 Da), IgG (MW 156,000 Da), BSA (66,330 Da), Ovalbumin (MW 43,000 Da), Peroxidase (40,200 Da), Beta lactoglobulin (MW18,400 Da), Myoglobin (MW 16,900 Da), Ribonuclease A (MW 13,700 Da), Cytochrome C (12,400 Da), Glycine tetramer (246 Da)
Columns: BioSuite 450, SEC, 7.5 x 300 mm, 13 µm; BioSuite 250, SEC, 7.5 x 300 mm, 13 µm; BioSuite 125, SEC, 7.5 x 300 mm, 10 µm
Part Numbers: 186002172 (BioSuite 450), 186002170 (BioSuite 250) & 186002168 (BioSuite 125)
Eluent: 0.1 M sodium phosphate, pH 7.0 containing 0.3 M sodium chloride
Flow Rate: 1.0 mL/min
Column Temp.: 25 °C
Detection: UV @ 220 nm

Column	Globular Protein MW Range	Branched Dextrans	Linear PEG/PEO
BioSuite 125	5,000–150,000	1,000–30,000	500–15,000
BioSuite 250	10,000–500,000	2,000–70,000	1,000–35,000
BioSuite 450	20,000–1,000,000	4,000–500,000	2,000–250,000

BioSuite Columns

Description	Matrix	Diameter Width	Diameter Length	Column Volume	Suggested Volume Load for Maximum Multicomponent Resolution*	Multicomponent Resolution*	Part No.
BioSuite 125, 4 µm UHR SEC	Silica	4.6 mm	300 mm	4.98 mL	Less than 8 mg/mL	Less than 40 µL	186002161
BioSuite 250, 4 µm UHR SEC	Silica	4.6 mm	300 mm	4.98 mL	Less than 8 mg/mL	Less than 80 µL	186002162
BioSuite UHR Guard SEC	Silica	4.6 mm	35 mm	—	—	—	186002163
BioSuite 125, 5 µm HR SEC	Silica	7.8 mm	300 mm	14.33 mL	Less than 8 mg/mL	Less than 200 µL	186002164
BioSuite 250, 5 µm HR SEC	Silica	7.8 mm	300 mm	14.33 mL	Less than 8 mg/mL	Less than 200 µL	186002165
BioSuite 450, 8 µm HR SEC	Silica	7.8 mm	300 mm	14.33 mL	Less than 8 mg/mL	Less than 200 µL	186002166
BioSuite HR Guard SEC	Silica	6 mm	40 mm	—	—	—	186002167
BioSuite 125, 10 µm SEC	Silica	7.5 mm	300 mm	13.25 mL	Less than 8 mg/mL	Less than 200 µL	186002168
BioSuite 125, 13 µm SEC	Silica	21.5 mm	300 mm	108.9 mL	Less than 8 mg/mL	Less than 1.6 mL	186002169
BioSuite 250, 10 µm SEC	Silica	7.5 mm	300 mm	13.25 mL	Less than 8 mg/mL	Less than 200 µL	186002170
BioSuite 250, 13 µm SEC	Silica	21.5 mm	300 mm	108.9 mL	Less than 8 mg/mL	Less than 1.6 mL	186002171
BioSuite 450, 13 µm SEC	Silica	7.5 mm	300 mm	13.25 mL	Less than 8 mg/mL	Less than 200 µL	186002172
BioSuite 450, 17 µm SEC	Silica	21.5 mm	300 mm	108.9 mL	Less than 8 mg/mL	Less than 1.6 mL	186002173
BioSuite Guard SEC	Silica	7.5 mm	75 mm	—	—	—	186002174
BioSuite Guard SEC	Silica	21.5 mm	75 mm	—	—	—	186002175

** Using a BSA protein standard in a 50 mM phosphate buffer containing salt (either 0.1 M NaCl or 0.1 M Na_2SO_4) eluent. Useful protein mass loads will vary depending upon separation eluent, complexity of sample, and on the type of proteins contained in mixture. In general, maximum component resolution is obtained by injecting the smallest possible volume of a dilute protein solution.*

** Note: Operating flow rates for BioSuite Ultra-High Resolution (UHR) SEC Columns (4.6-mm ID) are from 0.1–0.4 mL/min. Use of an HPLC system (e.g. Waters Alliance HPLC System) capable of operating at these flows is essential for optimal UHR SEC Column performance.*

Protein-Pak and Shodex Size-Exclusion HPLC Columns

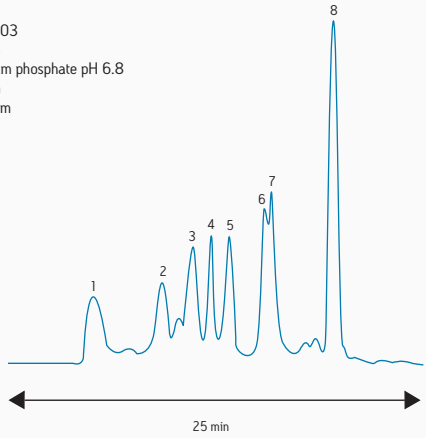
Waters offers two families of packings for size-exclusion chromatography. Protein-Pak™ packings are based on a 10 µm diol-bonded silica and are available in a selection of pore sizes and column configurations. In addition, Waters offers a series of Shodex 7 µm high-resolution, gel-filtration packings.

The Protein-Pak size-exclusion columns can be expected to resolve proteins that differ in molecular weight by a factor of two and to distinguish proteins differing by as little as 15% in molecular weight. The degree of resolution is more dependent on the sample mass and volume than the interaction between the sample and the stationary phase. Ideally, there should be no interaction between the stationary phase and the sample molecules. Secondary interactions are most often ionic and can, therefore, be reduced by increasing the ionic strength of the mobile phase. Typical, salt concentrations range to 0.2–0.5 M NaCl. It may also be useful in some cases to consider adding 10–20% methanol to eliminate hydrophobic and other hydrogen-bonding

Standard Protein Mix on KW-803 Column

Column: Protein KW-803
Part Number: WAT035946
Eluent: 25 mM sodium phosphate pH 6.8
Flow Rate: 0.72 mL/min
Detection: UV @ 280 nm

Compounds:
1. Blue dextran
2. Ferritin
3. Aldolase
4. Bovine serum albumin
5. Ovalbumin
6. Chymotrypsinogen
7. Cytochrome c
8. Cytidine



This gel-filtration separation of protein standards demonstrates the ability to separate proteins in a wide range of molecular weights in minutes for high sensitivity analysis or protein isolation up to the milligram scale.

Shodex Size-Exclusion Columns

Column	Particle Size	Dimension	MW Range	Part No.
Protein KW-802.5	7 µm	8 x 300 mm	100–50,000	WAT035943
Protein KW-803	7 µm	8 x 300 mm	100–150,000	WAT035946
Protein KW-804	7 µm	8 x 300 mm	500–600,000	WAT036613
Protein-Pak 125 Sentry Guard Column 3.9 x 20 mm, 2/pkg (requires holder)				186000926
Sentry Universal Guard Column Holder				WAT046910

Protein-Pak Columns and Packings

Steel Column	Dimension	MW Range	Part No.
Protein-Pak 60	7.8 x 300 mm	1,000–20,000	WAT085250
Protein-Pak 125	7.8 x 300 mm	2,000–80,000	WAT084601
Protein-Pak 300SW	7.5 x 300 mm	10,000–300,000	WAT080013
Protein-Pak 125 Sentry Guard Column 3.9 x 20 mm, 2/pkg (requires holder)			186000926
Sentry Universal Guard Column Holder			WAT046910
Protein-Pak 200SW	8 x 300 mm	500–60,000	WAT011786
Protein-Pak 300SW	8 x 300 mm	10,000–300,000	WAT011787

Inquire for additional offerings, including prep.

Waters is a leading manufacturer of analytical instrumentation and consumable products. We understand the importance of autosampler vials for the performance of analytical instrumentation. There are many factors to consider in selecting the proper vial:

- Needle design
- Autosampler tray design
- Chemical compatibility
- Cleanliness
- Optic and robotic specifications
- Volatility
- Sample volume

At Waters, we take all of these factors into consideration in the design, manufacture, and delivery of our vials and accessories. Unlike our competition, who offer Type I, 33-expansion glass in North America and Type I, 51-expansion glass in Europe or Japan, Waters single source manufacturing produces Type I, 33-expansion glass, the lowest free ion glass available, for worldwide distribution.

LC/GC Certified Vials

LC/GC Certified Vials are tested by HPLC using UV detection. The HPLC test was developed to look for trace levels of chemicals used in the manufacturing and packaging process. These chemicals include lubricants, surfactants, antistatic, and antioxidants from packaging. The tests are run on each batch of vials, after they have been packaged for several days, to ensure cleanliness. An additional head-space GC test is done to look for proper curing of the silicone septa.

TruView LCMS Certified Vials

TruView™ LCMS Certified Vials include the stringent dimensional tolerances and UV and MS cleanliness tests required of the LC/GC and LCMS Certified Vials lines. The additional product attribute of TruView vials is the glass surface exhibits low polar analyte adsorption. The vials are manufactured under tightly controlled process conditions (patent pending) that limit the concentration of free ions on glass surface. Low levels of free ions on the surface of glass can cause analyte adsorption. Waters TruView LCMS Certified Vials are tested for high recovery of analyte at 1 ng/mL concentration using UPLC/MS/MS (MRM) and yield little adsorption. These vials exhibit the lowest adsorption of autosampler vials in the market.

Choosing the Right Vial and Septum for Your Application

There are three decisions you need to make when choosing the correct vial for your application: the septum, the closure, and the vial itself. Read through the selection options below to determine the proper combination for your application. For your convenience, Waters offers many of these choices as combination packs. The vial, cap, and septum come pre-packaged as packs of 100 for ease and convenience in ordering.

PTFE

- Recommended for single injection applications
- Ideal for use in MS applications
- Excellent solvent resistance and chemical compatibility
- Does not reseal upon puncturing
- Not recommended for long-term sample storage

PTFE/Silicone

- Demonstrates excellent resealing characteristics
- PTFE chemical resistance until punctured, then the septum will have the chemical compatibility of silicone
- Working temperature range from -40 °C to 200 °C

Vial Closures Guide

Vials are available in three closure types: crimp, snap, and screw cap. Each closure has its advantages and disadvantages.

Crimp caps squeeze the septum between the rim of the glass vial and the crimped aluminum cap. This forms an excellent seal preventing
evaporation. The septum stays seated during piercing by the autosampler needle. The crimp cap vial requires crimping tools to carry out the
sealing process. For few samples, manual crimper tools are the best choice. For large numbers of samples, automated crimpers are available.

Snap caps are an extension of the crimp cap system of sealing. A plastic cap is stretched over the rim of the vial to form a seal by squeezing the septum between the glass and the stretched plastic cap. The plastic cap creates tension when trying to return to its original size. This tension forms the seal between glass, cap and septum. Plastic snap caps do not require any tools to assemble.

Snap caps are not as effective a seal as other closures.

- If the fit of the cap is very tight, the cap is hard to apply and may be subject to crack.
- If the fit is too loose, the seal is poor and the septum may dislodge.

LectraBond™ Screw Caps are available through Waters. This screw cap has a PTFE/silicone septum bonded to the polyethylene cap, using a non-solvent bonding process. This bonding technology is designed to keep the septum/cap together during shipment and assembly onto vials. The bond will aid in preventing dislodging of the septum during use, but the primary sealing mechanism is the mechanical force applied by tightening the cap to the vial.

Cap tightening is the mechanism that forms the seal and holds the septum in place during needle insertion. There is no need to over-tighten the cap, as it can compromise the seal and lead to dislodging. The septum starts to cup or indent when you begin to over-tighten.

Cap Design	Strength Design	Comment
Crimp	Excellent seal	Requires tools
Snap	Moderate seal	Fast, no tools, some cap cracking
Screw	Excellent seal	Universal

Screw Cap 12 x 32 mm Vials for Alliance Systems

	Clear	Amber	Max Recovery	Amber Max	Total Recovery
					
TruView LCMS Certified Combination Packs	**Part No.**	**Part No.**	**Part No.**	**Part No.**	**Part No.**
Vial, Cap, and Silicone/PTFE Septum	186005660CV	186005667CV	186005668CV	186005664CV	186005669CV
LC/GC Certified Combination Packs					
Bonded Silicone/PTFE Septum	186000272C	186000846C	186000326C	186003885C	186000384C
Bonded Pre-Slit Silicone/PTFE Septum	186000307C	186000847C	186000327C	186003886C	186000385C
Injectable Volumes Alliance 2690/2695					
Max	1100 µL	1100 µL	—	—	950 µL
Residual	750 µL	750 µL	—	—	9 µL
Injectable Volumes Alliance 2790/2795/2707					
Max	1700 µL	1700 µL	1500 µL	1500 µL	—
Residual	170 µL	170 µL	22 µL	22 µL	—
Inserts					
150 µL with Poly Spring	WAT094171(DV)[1]	WAT094171(DV)[1]	—	—	—
Max Volume Injection/Max Residual Volume	144 µL/6 µL	144 µL/6 µL	—	—	—
Compatible Systems					
Alliance 2690/2695	✓	✓			✓
Alliance 2790/2795/2707	✓	✓	✓	✓	

All items come in quantities of 100, unless otherwise noted.

This table highlights the most commonly used vials for GPC analysis. For a complete listing of Waters vial products refer to the Waters Quality Parts, Chromatography Columns and Supplies Catalog, www.waters.com/catalog.

GPC 2000 Vials

	4 mL Screw Cap	10 mL Screw Neck
		
Components	**Part No.**	**Part No.**
Vial	186000840	186001420
Black Screw Cap	600000162	186001421
PTFE Septum	WAT072714[1]	186001422

[1] Item contains 144 vials.

Vials for Waters Breeze with 717 Autosampler

	4 mL Screw Neck	Amber Screw Neck	Total Recovery
15 x 45 mm Vials			
Combination Packs	**Part No.**	**Part No.**	**Part No.**
Vial, Cap, and LectraBond PTFE/Silicone Septum	186000838C	186001133C	186002629C
Vial, Cap, and LectraBond Pre-Slit PTFE/Silicone Septum	186000839C	186001134C	186002630C
Max Volume Injection/Max Residual Volume	2400 µL/1600 µL	2400 µL/1600 µL	3000 µL/40 µL
Insert	**Part No.**	**Part No.**	**Part No.**
250 µL Glass Insert[2]	WAT072704(DV)[1]	WAT072704(DV)[1]	—
Max Volume Injection/Max Residual Volume	244 µL/6 µL	244 µL/6 µL	—
Springs for LVI, 100/pk	WAT072708	WAT072708	—

[2] Inserts require springs (Part No. WAT072708).

These tables highlight the most commonly used vials for GPC analysis. For a complete listing of Waters vial products refer to the Waters Quality Parts, Chromatography Columns and Supplies Catalog, www.waters.com/catalog.

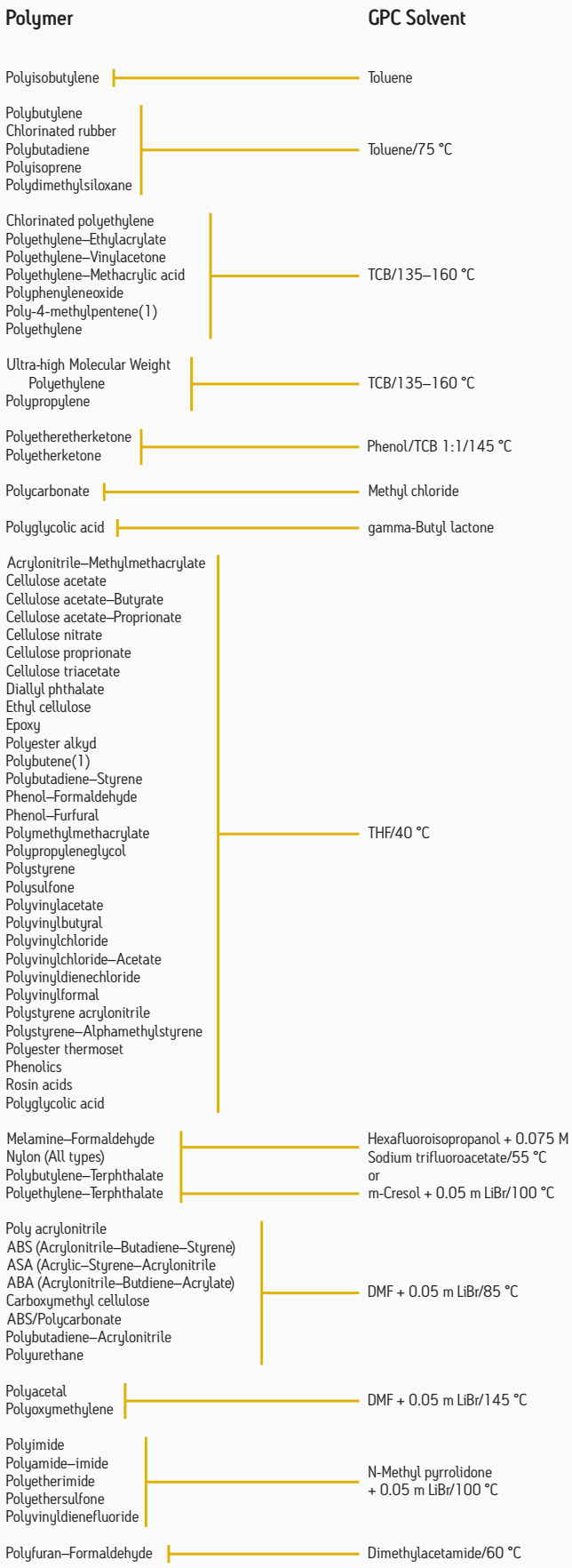
Solvent Considerations

One of the most important decisions for an analyst is finding a suitable solvent to dissolve the polymer for analysis. This may sound trivial, but remember that GPC is a separation technique based on the size of the polymer in solution. Polymer chains will open up to a certain relaxed conformation in solution, and the solvent chosen will determine what this size will be. Many polymers are soluble at room temperature in various solvents, but in some cases (especially for highly crystalline polymers) high temperature is required for dissolution. The following is a guide for both aqueous and non-aqueous soluble polymers.

Aqueous SEC Solvent Selection Guide

Polymer	Class	Eluent
Polyethylene oxide Polyethylene glycol Polysaccharides, Pullulans Dextrans Celluloses (water soluble) Polyvinyl alcohol Polyacrylamide	Neutral	0.10 M Sodium nitrate
Polyvinyl pyrrolidone	Neutral, hydrophobic	
Polystyrene sulfonate Lignin sulfonate	Anionic, hydrophobic	80:20 0.10 M Sodium nitrate/Acetonitrile
Collagen/Gelatin	Amphoteric	
Polyacrylic acid Polyalginic acid/alginates Hyaluronic acid Carrageenan	Anionic	0.10 M Sodium nitrate
DEAE dextran Polyvinylamine	Cationic	0.80 M Sodium nitrate
Polyepiamine	Cationic	0.10% TEA
n-Acetylglucosamine	Cationic	0.10 M TEA/1% Acetic acid
Polyethyleneimine Poly(n-methyl-2-vinyl pyridinium) I salt	Cationic, hydrophobic	0.50 M Sodium acetate/0.50 M Acetic acid
Lysozyme Chitosan	Cationic, hydrophobic	0.50 M Acetic acid/0.30 M Sodium sulfate
Polylysine	Cationic, hydrophobic	5% Ammonium biphosphate/3% Acetonitrile (pH = 4.0)
Peptides	Cationic, hydrophobic	0.10% TFA/40% Acetonitrile

Non-Aqueous GPC Solvent Selection Guide



Frequently Asked Questions

What solvent should I buy my columns packed in, and why?

Non-Aqueous GPC columns are packed in either:

- THF
- Toluene
- DMF

Specialty columns packed in methanol specifically for analysis at room temperature with HFIP (hexafluoroisopropanol) are available. If you are using a solvent other than these four for your application, there are a couple of rules-of-thumb to think about. If you are doing a "room temperature" application in a solvent such as chloroform or methylene chloride, convert over from THF. If you plan on doing high-temperature work in TCB, ODCB, for example, convert over from toluene at ~85 - 90 °C. If you are going to use a solvent that is very polar, such as DMAC (Dimethyl-acetamide) or NMP (n-methylpyrollidone), convert over from DMF.

I currently have columns in solvent "A", can I switch to solvent "B"?

Generally, one can switch directly from one solvent to another at 0.1 - 0.2 ml/min if the two solvents are miscible (refer to the column's care and use manual). If the solvents are not miscible, an intermediate solvent (which both solvents are miscible in) will have to be used.

What additives are important and when should I use them?

In certain cases, some mobile-phase additive is required. For example, 0.05 M lithium bromide is added to polar solvents such as DMF, DMAC and NMP. These polar solvents are used to analyze polar polymers such as polyurethanes or polyimides, and there is a dipole interaction that occurs, causing artificial shoulders to appear on the high molecular weight end of the distribution. This interaction is eliminated with the addition of the salt.

Salts are also used in aqueous GPC as the methacrylate gels used in the columns have an overall anionic charge. Due to this charge, ion exclusion can occur with anionic samples and ion absorption can occur with cationic samples. The use of sodium nitrate salt can minimize these effects as well as pH adjustment of the eluent if any ion-exchange interaction is occurring with cationic samples.

In which order should I place the columns, and why?

Generally, it does not matter what order the columns are placed in. The order will not affect the molecular weight distribution calculations of the eluting polymer. It is a good idea, however, to always place the 50 Å or 100 Å columns at the end of the set, as the styrene/divinylbenzene gel in these columns tend to be softer and less durable.

What flow rate should I use in my GPC column?

It is recommended not to exceed 1.0 mL/min for the 7.8 mm ID analytical columns. The "optimum" resolution for these columns is approximately 0.70 to 0.80 mL/min. The optimum flow rate for the 4.6 mm ID narrow-bore columns is 0.3 to 0.35 mL/min. Refer to the column's care and use manual for more details.

When starting up columns, should I gradually increase flow and temperature?

It is mandatory to slowly ramp up the flow rate for analytical GPC columns, particularly the Stryagel HR series. Sudden increase in flow (and subsequently pressure) will certainly damage the columns. Temperature ramping is not as critical. Generally, we ramp the flow rate from 0.0 to
1.0 mL/min over a 60-second interval, and the temperature from ambient to 150 °C (as an example) over several hours.

How do I choose the column's pore size range?

The range of pore sizes is chosen by determining the approximate molecular weight range of the sample of interest. Choosing columns that target the molecular weight range of the polymer will provide the highest resolution. For example, if the polymer molecular weight range is low then a column set of 50, 500 and 1000 Å would be used; a medium molecular weight range requires a larger pore distribution so a 1000, 10,000, and 100,000 Å column set would be appropriate. For an unknown molecular weight range it is a good idea to use mixed bed (i.e. "linear", or "extended range") columns that provide a mixture of pore sizes..

The following table lists the molecular weight range of separation for individual pore size columns of styrene/divinylbenzene packings, based on polystyrene chain length exclusion limits.

MW Range	Pore Size	MW Range	Pore Size
100 - 1000	50 Å	50,000 - 1,000,000	100,000 Å
250 - 2500	100 Å	200,000 - > 5,000,000	1,000,000 Å
1,000 - 18,000	500 Å	500,000 - ~20,000,000	10,000,000 Å
5,000 - 40,000	1000 Å	~1,000 - 10,000,000	Mixed Bed - High
10,000 - 200,000	10,000 Å	~100 - 100,000	Mixed Bed - Low

What is resolution? How much do I need?

In GPC analysis, resolution means range of molecular weight separated in an incremental volume of elution. We would like to maximize this whenever possible. The easiest way to maximize this is to add more columns (and therefore analysis time, unfortunately). Another way is to use a smaller particle size (~ 5 µm), which will increase efficiency. The trade-off here is column durability and lifetime. In separations where oligomers, additives, and multi-modal distributions are present, resolution may be important. If the sample is a high density polyethylene with a broad distribution, resolution may not be as important.

Waters manufactures columns in the high resolution range (HR series) which contain 5-µm particles, the HT series which have ~ 10-µm particles (good for high temperature work and multiple solvent changeovers), and the HMW series that have 20-µm particles. These are good for very high molecular weight samples where shearing is a problem and resolution is not as critical.

Tip: The GPC solvent guide provides typical operating temperature ranges. For GPC analysis, columns are heated (even for room temperature applications) to increase resolution by improving analyte permeation.

What is a "narrow" standard? What is a "broad" standard?

- Narrow standards are those where the polydispersity is less than ~1.10. The polydispersity is defined as the ratio of the weight average molecular weight (Mw) to the number average molecular weight (Mn).
- Broad standards have polydispersities greater than 1.10 and are usually the same polymer as the sample to be analyzed.

If I use narrow standards, can I inject more than one standard at a time?

In conventional GPC with RI detection, it is certainly acceptable to inject a mixture of standards, as long as there is sufficient resolution among the eluted standards. We would suggest a maximum of three. With advanced detection, such as viscometry, where the area under the curve for the standard needs to be known accurately, one standard at a time should be injected.

What standard(s) should I use for my polymer?

For most people, a narrow standard relative calibration is fine. In this case, polystyrene standards are the usual choice for organic GPC, but PMMA's, polyisoprenes, polybutadienes and polyTHF narrow standards may be used. For aqueous GPC, narrow polyethylene oxides, polyethylene glycols and pullulans (polysaccharides) are available. If the user needs the true molecular weight (relative to the calibrant not being good enough), the broad standard (or reference) with the same chemical nature as the samples may be used.

SALES OFFICES:

Austria 43 1 877 18 07

Australia 61 2 9933 1777

Belgium and Luxembourg 32 2 726 1000

Brazil 55 11 4134 3788

Canada 1 800 252 4752

China 86 21 6156 2666

Czech Republic 420 2 617 11384

Denmark 45 46 59 8080

Finland 358 9 5659 6288

France 33 1 30 48 72 00

Germany 49 6196 400 600

Hong Kong 852 2964 1800

Hungary 36 1 350 5086

India 91 080 49292200 03

Ireland 353 1 448 1500

Israel 9723 3731391

Italy 39 02 265 0983

Japan 81 3 3471 7191

Korea 82 2 6300 9200

Mexico 52 55 52 00 1860

The Netherlands 31 76 508 7200

Norway 47 6 384 6050

Poland 48 22 101 5900

Portugal 351 21 893 61 77

Puerto Rico 1 787 747 8445

Russia/CIS 7 495 727 4490 / 290 9737

Singapore 65 6593 7100

Spain 34 93 600 9300

Sweden 46 8 555 115 00

Switzerland 41 56 676 7000

Taiwan 886 2 2501 9928

UK 44 208 238 6100

US 1 800 252 4752

www.waters.com/gpc

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 508 478 2000
F: 508 872 1990
www.waters.com

August 2014 720004331EN KP-FP