

Solutions for Aggregate and Fragment Analyses

Size-exclusion chromatography (SEC) is a powerful technique for aggregate and fragment analysis in the research, development, and manufacturing of biotherapeutic proteins, as well as viral vectors.

Important Considerations in peptides and SEC Column Selection

What is the molecular weight of your sample?

MW 1-80K Da

MW 10-450K/650K Da

MW 100-1500K Da

Pore Size Recommendation

125 Å

200 Å/250 Å*

450 Å

What is the dispersion of your LC system? **

<20 µL (UPLC™)

>20-≤35 µL (UHPLC)

>35 µL (HPLC)

Particle Size Recommendation

1.7 µm

2.5 µm

2.5 µm

Are you separating components <2-fold difference in MW? ***

1.7 µm

2.5 µm

2.5 µm

Column Dimension Recommendation

4.6 x 300 mm

7.8 x 300 mm

7.8 x 300 mm

Are you separating components ≥2-fold difference in MW?

1.7 µm

2.5 µm

2.5 µm

Column Dimension Recommendation

4.6 x 150 mm

7.8 x 150 mm

7.8 x 150 mm

*Available in MaxPeak™ Premier hardware to reduce undesired secondary interactions.

**System dispersion measures at 5σ peak width. For guidance on measuring system dispersion, download the SEC Optimization Guide (p/n: 720006067EN) on Waters.com.

***Larger column length provides more resolution. To understand the "why" behind these recommendations, read the Application Note (p/n: 720006336EN) on Waters.com.

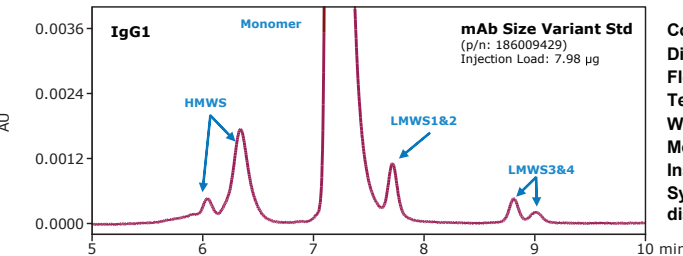
SEC COLUMN TECHNOLOGY THAT PROVIDES:

- Fewer non-desired secondary interactions for reliable decision-making data
- Enhanced stability at pH values >7 for SEC method flexibility
- Scalable chemistries for use in product discovery, development, and manufacturing
- Consistent performance due to comprehensive QC testing with relevant protein or mAb Size Variant Standards

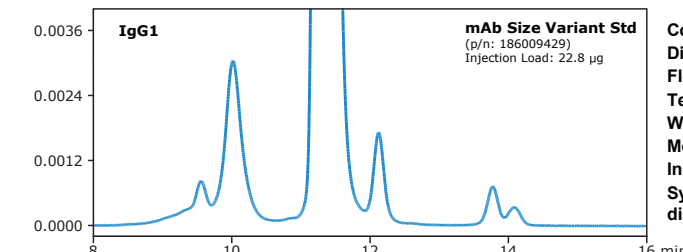
MAXPEAK™
PREMIER

BEH Technology™

Scalable SEC Column Offerings using 2x PBS Mobile Phase



Column: ACQUITY™ Premier Protein SEC 250 Å, 1.7 µm
Dimension: 4.6 x 300 mm
Flow rate: 300 µL/min
Temp.: 35 °C
Wavelength: 280 nm
Mobile phase: 2x PBS (20 mM phosphate, 276 mM NaCl, 5.4 mM KCl pH 7.4)
Instrument: ACQUITY UPLC H-Class Bio
System dispersion: <10 µL



Column: XBridge™ Premier Protein SEC 250 Å, 2.5 µm
Dimension: 7.8 x 300 mm
Flow rate: 575 µL/min
Temp.: 35 °C
Wavelength: 280 nm
Mobile phase: 2x PBS (20 mM phosphate, 276 mM NaCl, 5.4 mM KCl pH 7.4)
Instrument: ACQUITY UPLC H-Class Bio
System dispersion: 30 µL

Ordering Information

Molecular Weight Range		1.7 µm Particle Size	
125 Å Pore Size; 1000–80,000 Daltons (e.g., Peptides and Small Proteins)			
<20 µL LC dispersion/components >2x size differences	ACQUITY UPLC Protein BEH 125 Å, 1.7 µm	4.6 x 150 mm with BEH125 Protein Standard Mix	176003906
<20 µL LC dispersion/components <2x size differences		4.6 x 300 mm with BEH125 Protein Standard Mix	176003907
>20 µL but <35 µL LC dispersion/components >2x size differences	ACQUITY UPLC Protein BEH 125 Å, 1.7 µm	4.6 x 300 mm with BEH125 Protein Standard Mix	176003907
>20 µL but <35 µL LC dispersion/components <2x size differences		Not recommended	—
>35 µL LC dispersion/components >2x size differences	ACQUITY UPLC Protein BEH 125 Å, 1.7 µm	4.6 x 300 mm with BEH125 Protein Standard Mix	176003907
>35 µL LC dispersion/components <2x size differences		Not recommended	—
200 Å/250 Å Pore Size; 10,000–450,000/650,000 Daltons (e.g., mAbs, ADCs, Bi-specifics)			
<20 µL LC dispersion/components >2x size differences	ACQUITY Premier Protein BEH 250 Å, 1.7 µm	4.6 x 150 mm with mAb Size Variant Standard Mix	176005071
<20 µL LC dispersion/components <2x size differences		4.6 x 300 mm with mAb Size Variant Standard Mix	176005072
>20 µL but <35 µL LC dispersion/components >2x size differences	ACQUITY Premier Protein BEH 250 Å, 1.7 µm	4.6 x 300 mm with mAb Size Variant Standard Mix	176005072
>20 µL but <35 µL LC dispersion/components <2x size differences		Not available	—
>35 µL LC dispersion/components >2x size differences	ACQUITY Premier Protein BEH 250 Å, 1.7 µm	4.6 x 300 mm with mAb Size Variant Standard Mix	176005072
>35 µL LC dispersion/components <2x size differences		Not available	—
450 Å Pore Size; 450,000–1,500K Daltons (e.g., AAVs, IgMs)			
<20 µL LC dispersion/components >2x size differences	ACQUITY UPLC Protein BEH 450 Å, 2.5 µm	4.6 x 150 mm with BEH450 Protein Standard Mix	176002996
<20 µL LC dispersion/components <2x size differences		4.6 x 300 mm with BEH450 Protein Standard Mix	176002997
>20 µL but <35 µL LC dispersion/components >2x size differences	ACQUITY UPLC Protein BEH 450 Å, 2.5 µm	4.6 x 300 mm with BEH450 Protein Standard Mix	176002997
>20 µL but <35 µL LC dispersion/components <2x size differences		Not available	—
>35 µL LC dispersion/components >2x size differences	ACQUITY UPLC Protein BEH 450 Å, 2.5 µm	4.6 x 300 mm with BEH450 Protein Standard Mix	176002997
>35 µL LC dispersion/components <2x size differences		Not available	—
Molecular Weight Range		2.5 µm Particle Size	
125 Å Pore Size; 1000–80,000 Daltons (e.g., Peptides and Small Proteins)			
<20 µL LC dispersion/components >2x size differences	XBridge Protein BEH 125 Å, 2.5 µm	4.6 x 150 mm with BEH125 Protein Standard Mix	176004332
<20 µL LC dispersion/components <2x size differences		4.6 x 300 mm with BEH125 Protein Standard Mix	176004323
>20 µL but <35 µL LC dispersion/components >2x size differences	XBridge Protein BEH 125 Å, 2.5 µm	7.8 x 150 mm with BEH125 Protein Standard Mix	176004323
>20 µL but <35 µL LC dispersion/components <2x size differences		4.6 x 300 mm with BEH125 Protein Standard Mix	176004324
>35 µL LC dispersion/components >2x size differences	XBridge Protein BEH 125 Å, 2.5 µm	7.8 x 150 mm with BEH125 Protein Standard Mix	176004323
>35 µL LC dispersion/components <2x size differences		4.6 x 300 mm with BEH125 Protein Standard Mix	176004324
200 Å/250 Å Pore Size; 10,000–450,000/650,000 Daltons (e.g., mAbs, ADCs, Bi-specifics)			
<20 µL LC dispersion/components >2x size differences	XBridge Premier Protein SEC 250 Å, 2.5 µm	4.6 x 150 mm with mAb Size Variant Standard Mix	176005067
<20 µL LC dispersion/components <2x size differences		4.6 x 300 mm with mAb Size Variant Standard Mix	176005068
>20 µL but <35 µL LC dispersion/components >2x size differences	XBridge Premier Protein SEC 250 Å, 2.5 µm	7.8 x 150 mm with mAb Size Variant Standard Mix	176005069
>20 µL but <35 µL LC dispersion/components <2x size differences		7.8 x 300 mm with mAb Size Variant Standard Mix	176005070
>35 µL LC dispersion/components >2x size differences	XBridge Premier Protein SEC 250 Å, 2.5 µm	7.8 x 150 mm with mAb Size Variant Standard Mix	176005069
>35 µL LC dispersion/components <2x size differences		7.8 x 300 mm with mAb Size Variant Standard Mix	176005070
450 Å Pore Size; 450,000–1,500K Daltons (e.g., AAVs, IgMs)			
<20 µL LC dispersion/components >2x size differences	XBridge Protein BEH SEC 450 Å, 2.5 µm	4.6 x 150 mm	186009179
<20 µL LC dispersion/components <2x size differences		4.6 x 300 mm	186009180
>20 µL but <35 µL LC dispersion/components >2x size differences	XBridge Protein BEH SEC 450 Å, 2.5 µm	Not available	—
>20 µL but <35 µL LC dispersion/components <2x size differences		Not available	—
>35 µL LC dispersion/components >2x size differences	XBridge Protein BEH SEC 450 Å, 2.5 µm	Not available	—
>35 µL LC dispersion/components <2x size differences		Not available	—
Molecular Weight Range		3.5 µm Particle Size	
125 Å Pore Size; 1000–80,000 Daltons (e.g., Peptides and Small Proteins)			
<20 µL LC dispersion/components >2x size differences	XBridge Protein BEH SEC 125 Å, 3.5 µm	7.8 x 150 mm with BEH125 Protein Standard Mix	176003592
<20 µL LC dispersion/components <2x size differences		7.8 x 300 mm with BEH125 Protein Standard Mix	176003592
>20 µL but <35 µL LC dispersion/components >2x size differences	XBridge Protein BEH SEC 125 Å, 3.5 µm	7.8 x 150 mm with BEH125 Protein Standard Mix	176003592
>20 µL but <35 µL LC dispersion/components <2x size differences		7.8 x 300 mm with BEH125 Protein Standard Mix	176003593
>35 µL LC dispersion/components >2x size differences	XBridge Protein BEH SEC 125 Å, 3.5 µm	7.8 x 150 mm with BEH125 Protein Standard Mix	176003592
>35 µL LC dispersion/components <2x size differences		7.8 x 300 mm with BEH125 Protein Standard Mix	176003593
200 Å Pore Size; 10,000–450,000 Daltons (e.g., mAbs, ADCs, Bi-specifics)			
<20 µL LC dispersion/components >2x size differences	XBridge Protein BEH SEC 200 Å, 3.5 µm	7.8 x 150 mm with BEH200 Protein Standard Mix	176003595
<20 µL LC dispersion/components <2x size differences		7.8 x 300 mm with BEH200 Protein Standard Mix	176003595
>20 µL but <35 µL LC dispersion/components >2x size differences	XBridge Protein BEH SEC 200 Å, 3.5 µm	7.8 x 150 mm with BEH200 Protein Standard Mix	176003595
>20 µL but <35 µL LC dispersion/components <2x size differences		7.8 x 300 mm with BEH200 Protein Standard Mix	176003596
>35 µL LC dispersion/components >2x size differences	XBridge Protein BEH SEC 200 Å, 3.5 µm	7.8 x 150 mm with BEH200 Protein Standard Mix	176003595
>35 µL LC dispersion/components <2x size differences		7.8 x 300 mm with BEH200 Protein Standard Mix	176003596
450 Å Pore Size; 450,000–1,500K Daltons (e.g., AAVs, IgMs)			
<20 µL LC dispersion/components >2x size differences	XBridge Protein BEH SEC 450 Å, 3.5 µm	7.8 x 150 mm with BEH450 Protein Standard Mix	176003598
<20 µL LC dispersion/components <2x size differences		7.8 x 300 mm with BEH450 Protein Standard Mix	176003599
>20 µL but <35 µL LC dispersion/components >2x size differences	XBridge Protein BEH SEC 450 Å, 3.5 µm	7.8 x 150 mm with BEH450 Protein Standard Mix	176003598
>20 µL but <35 µL LC dispersion/components <2x size differences		7.8 x 300 mm with BEH450 Protein Standard Mix	176003599
>35 µL LC dispersion/components >2x size differences	XBridge Protein BEH SEC 450 Å, 3.5 µm	7.8 x 150 mm with BEH450 Protein Standard Mix	176003598
>35 µL LC dispersion/components <2x size differences		7.8 x 300 mm with BEH450 Protein Standard Mix	176003599
BEH125 SEC Protein Standard Mix			186006519
BEH200 SEC Protein Standard Mix			186006518
BEH450 SEC Protein Standard Mix			186006842
mAb Size Variant Standard			186009429

For a complete list of SEC products, visit [waters.com/SEC](https://www.waters.com/SEC)