Waters
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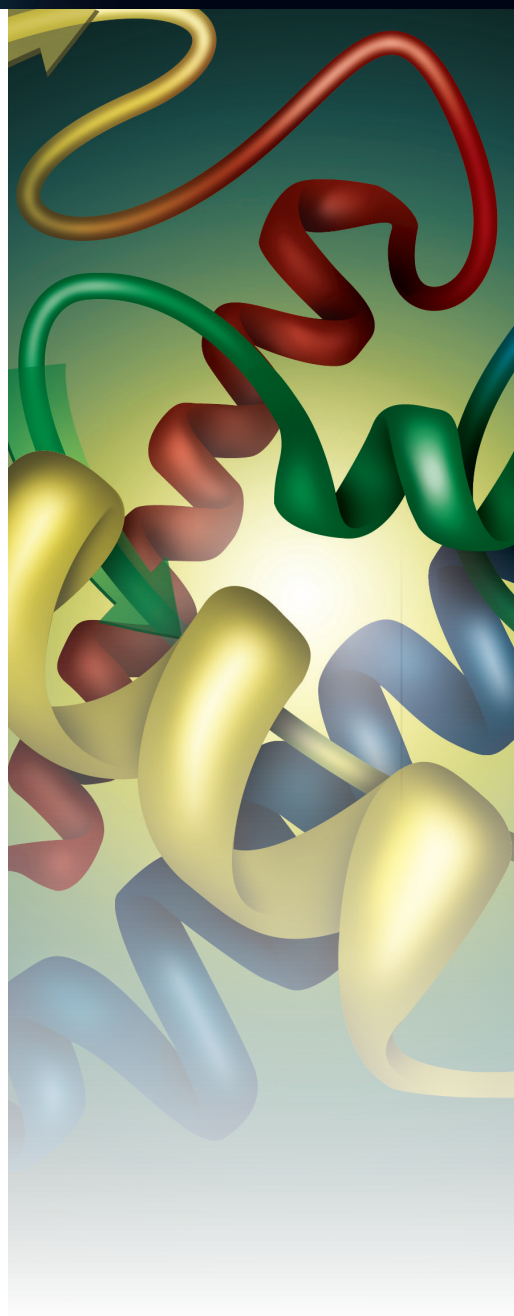
XBridge Protein BEH SEC Columns for HPLC-based Separations



Waters XBridge® Protein BEH SEC, 200Å and 450Å, 3.5 µm Columns were developed for use on HPLC instrumentation and to complement our existing line of smaller particle sized, UPLC®-based SEC columns for size-exclusion chromatography (SEC) of proteins. These new HPLC SEC columns are based on the same Waters ethylene bridged hybrid (BEH)-based particle technology with stable diol-bonding to deliver superior performance compared to traditional, 100% silica-based SEC offerings. Consequently, chromatographers now have the ability to easily develop and/or transfer SEC methods based on laboratory instrumentation, required protein component resolution, and sample throughput requirements.

- HPLC-based SEC resolution of proteins from 10–1,500K Daltons with higher throughput capability
- Outstanding SEC column life
- Less non-desired, protein/column interactions than silica-based SEC columns
- Comprehensive testing to provide unmatched column consistency and increased confidence in validated methods
- Complement ACQUITY UPLC®-based SEC columns for seamless method transfer based on application needs

HPLC-BASED SEC COLUMNS FOR PROTEIN SEPARATIONS

Reliable, high resolving, SEC methods are routinely used in the discovery, development, and quality assessment of protein-based biotherapeutics. Waters XBridge Protein BEH SEC, 200Å and 450Å, 3.5 µm Columns separate proteins from approximately to 10k to 1,500K Daltons. The diol-bonded XBridge Protein BEH SEC, 3.5 µm Columns are flow and pressure tolerant for increased sample throughput on HPLC systems compared to use of many traditional, silica-based SEC columns containing >5 µm particles.

Calibration Curves of Proteins and Peptides on XBridge Protein BEH SEC, 200Å and 450Å, 3.5 µm Columns

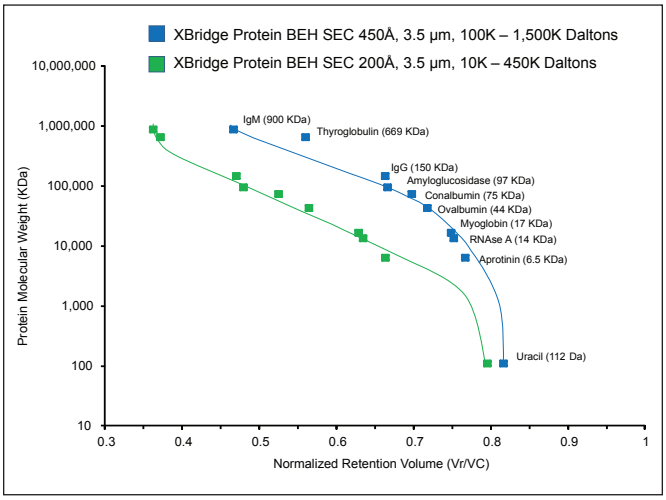


Figure 1. Calibration curves of various proteins, peptides, and uracil generated on the XBridge Protein BEH SEC 200Å (green) and 450Å (blue), 3.5 µm particle-size SEC Columns.

Separation of Protein and Peptide Standards on XBridge Protein BEH SEC, 200Å and 450Å, 3.5 µm Columns

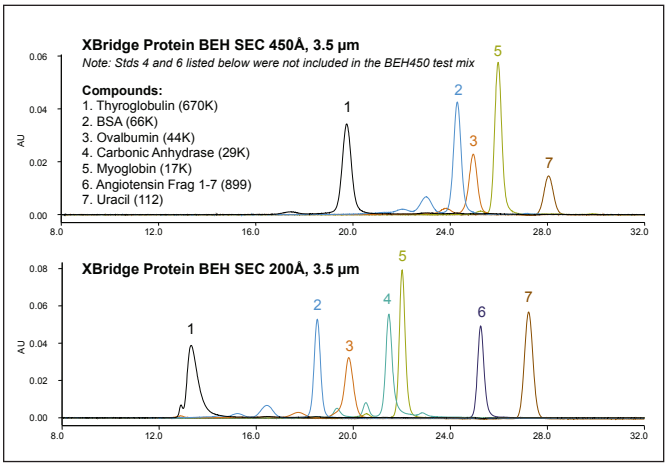


Figure 2. Comparative separations of curves of various proteins, peptides, and uracil on the XBridge Protein BEH SEC 450Å (top) and 200Å (bottom), 3.5 µm particle-size SEC Columns.

Higher Sample Throughput Using XBridge Protein BEH SEC 3.5 µm Columns

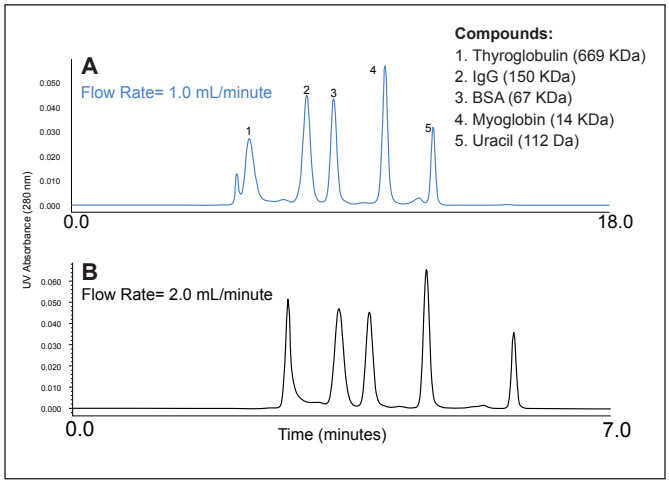


Figure 3. Comparative Separation of Waters BEH200 SEC Protein Standard Mixture (P/N: 186006518) on (A) competitor's 250Å, silica-based, 5 µm SEC column, flow rate 1.0 mL/minute and (B) XBridge Protein BEH SEC, 200Å, 3.5 µm Column, flow rate 2.0 mL/minute with 100 mM Sodium Phosphate Buffer, pH 6.8 mobile phase. Both column dimensions were 7.8 mm x 300 mm length and the same sample loads were injected. The time axis for the main chromatograms have been normalize. Peak identities for chromatograms A and B are: 1) thyroglobulin (669 KDa), 2) IgG (150 KDa), 3) BSA (67 KDa), 4) myoglobin (14 KDa), and uracil (112 Da).

Note: Comparable molecular weight standard profiles are observed, with the exception that the larger pore-size of the 250Å, 5 µm silica-based particles provide improved resolution of the thyroglobulin dimer peak (1.3 MDa) than what is observed on the 200Å, 3.5 µm BEH-based particle. Use of Waters XBridge Protein BEH SEC, 450Å, 3.5 µm is recommended for the analysis of proteins, such as thyroglobulin and its dimer, whose molecular weights exceed those recommended be analyzed on the XBridge Protein BEH SEC ,200Å, 3.5 µm Column.

OUTSTANDING SEC COLUMN LIFE WITH LESS NON-DESIRED INTERACTIONS USING BEH PARTICLE TECHNOLOGY

BEH Technology™ is well established for chromatography of various biological compounds with stability and performance attributes not found with many traditional, 100% silica-based particles (Anal Chem. 75 6781–6788 2003). The combination of the BEH base particle and innovative diol bonding process results in column stability, performance, and lifetime unheard of in traditional size-exclusion chromatographic columns. Also, compared to traditional silica-based SEC columns, less charged silanols exist on the diol-coated BEH particles contained in Waters XBridge Protein BEH SEC Columns which translates into less non-desirable ionic interactions between the protein and the SEC particle.

Waters XBridge Protein BEH SEC, 200Å, 3.5µm Column Performance Over 600 Injections

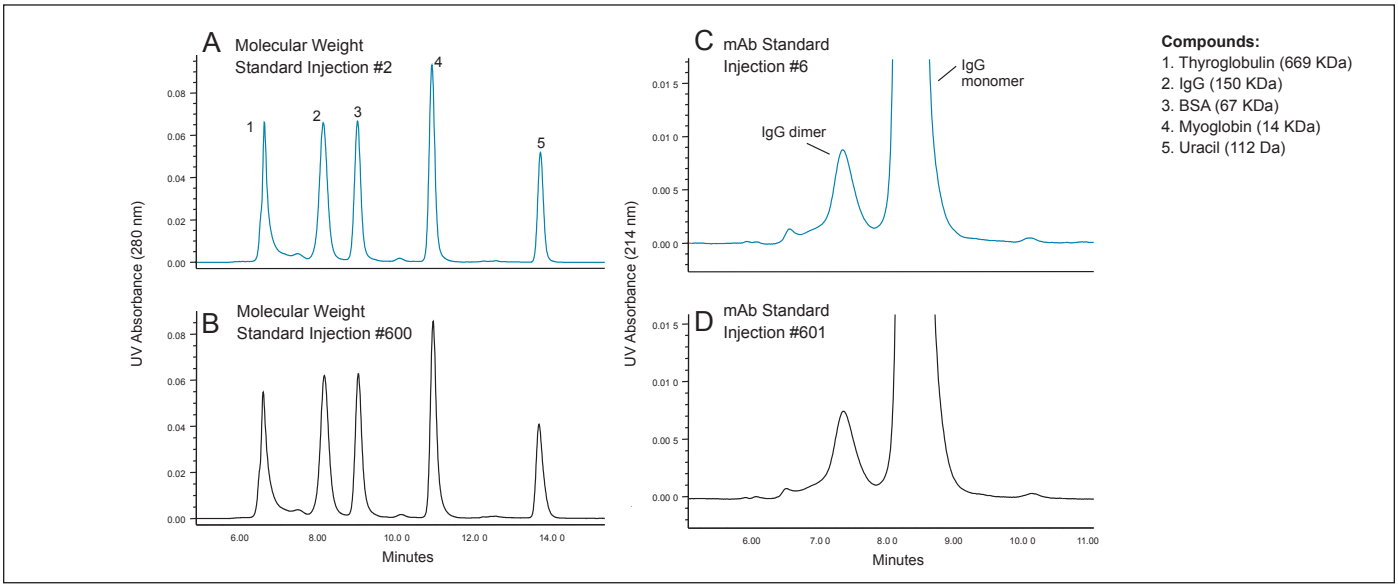


Figure 4. Column life time study using Waters BEH450 SEC Protein Standard (P/N 186006842) and Intact mAb Mass Check Standard (P/N 186006552, diluted to 1 mg/mL) on Waters XBridge Protein BEH SEC, 450Å, 3.5 µm, 7.8 x 300 mm Column. For the chromatograms of the mAb standard (C and D) the molecular weights of the IgG monomer and dimer are approximately 150 KDa and 300 KDa, respectively.

Effect of SEC Eluent Ionic Strength on the Analysis of the Basic Protein Lysozyme on 100% Silica vs. BEH SEC Particles

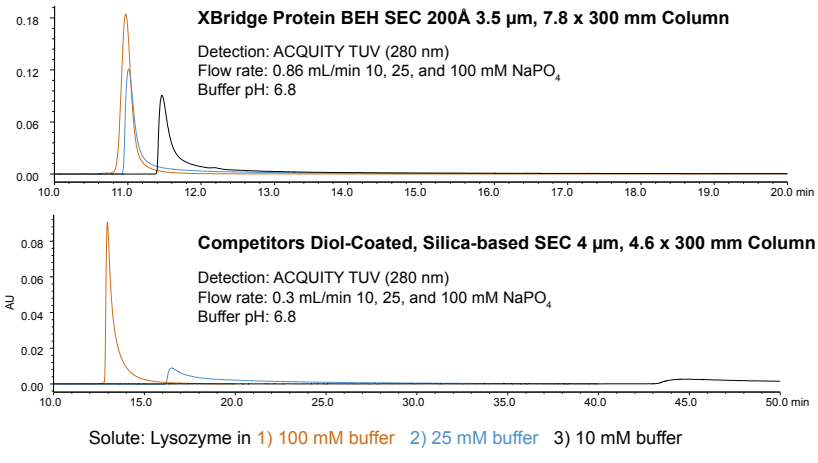


Figure 5. With many SEC columns containing silica-based, diol-bonded particles, undesirable secondary ionic interactions can occur between negatively-charged surfaces on the particle surface and basic proteins that can result in long retention times and excessive peak tailing. Traditionally, the solution frequently involves use of SEC eluents containing high concentrations of salt to minimize these ionic interactions. The unique BEH-diol particle surface on XBridge Protein BEH SEC, 200Å and 450Å Columns significantly reduces these secondary interactions, resulting in the ability to use less aggressive mobile phase salt concentrations.

STRINGENT MANUFACTURING QUALITY DELIVERS CONFIDENCE IN SEC GENERATED DATA

All Waters HPLC- and UPLC-based, BEH SEC particles are synthesized in state-of-the-art, ISO-certified manufacturing facilities from high quality raw materials, and are extensively QC tested throughout the synthetic process. In addition, each manufactured batch of XBridge Protein BEH SEC, 200Å and 450Å, 3.5 µm material is tested with relevant proteins to help ensure unmatched batch-to-batch consistency for supreme confidence in validated methods.

Batch-to-Batch and Column-to-Column Reproducibility on XBridge Protein BEH SEC Columns

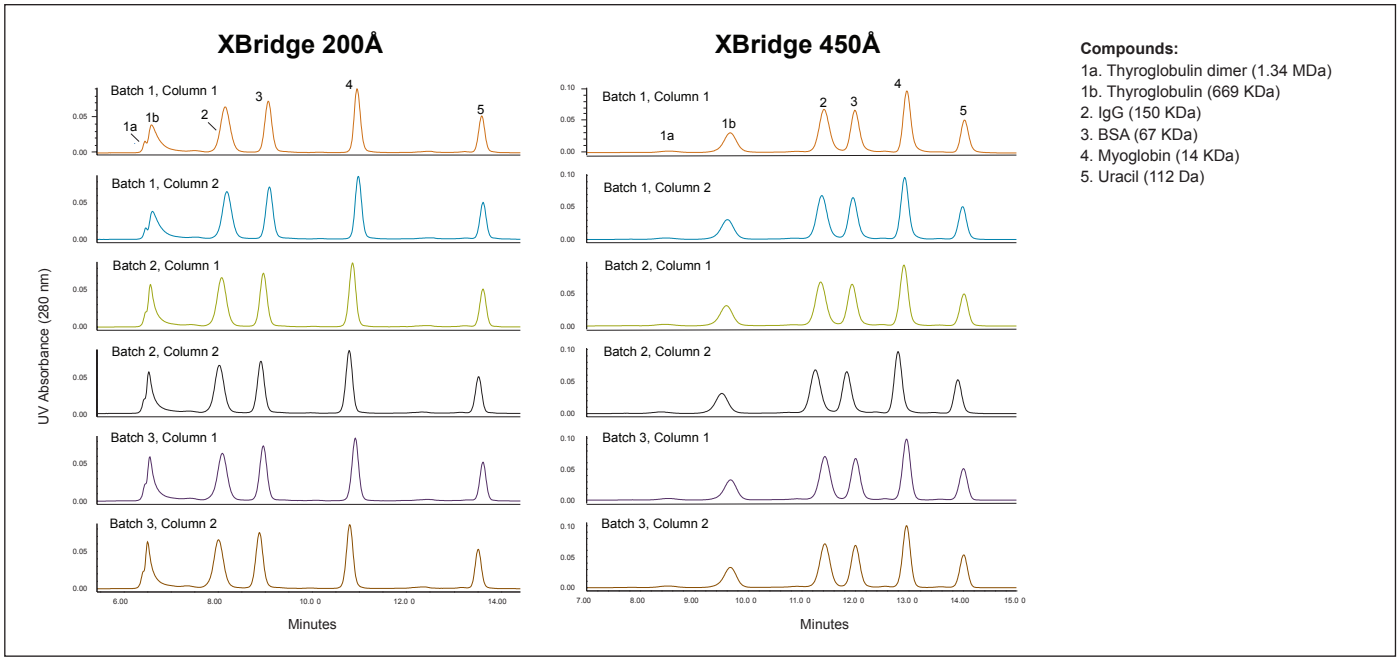
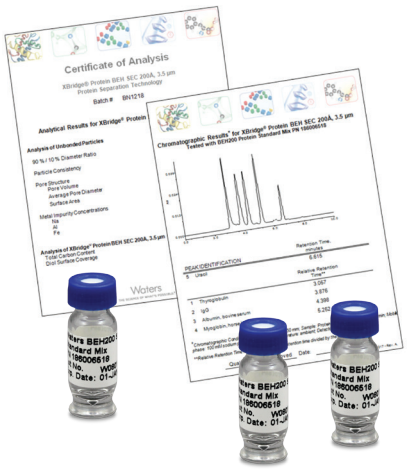


Figure 6. Separations of Waters BEH200 and BEH450 SEC Protein Standard Mixes on XBridge Protein BEH SEC, 200Å and 450Å, 3.5 µm, 7.8 mm x 300 mm Columns showing excellent manufacturing and column packing consistency. Two columns were packed from 3 different manufacturing batches of BEH-diol bonded particles to evaluate and confirm industry leading, column-to-column and batch-to-batch reproducibility.



BENCHMARKING, METHOD DEVELOPMENT, AND TROUBLESHOOTING: BEH200 OR BEH450 SEC PROTEIN STANDARDS

Each XBridge Protein BEH SEC, 200Å and 450Å, 3.5 µm Column is shipped with a lyophilized vial of the appropriate BEH SEC Protein Standard Mix. The same protein standard formulation is used by Waters manufacturing to ensure SEC Column batch-to-batch consistency. Consequently, chromatographers can now use these same materials to benchmark a new SEC column or troubleshoot issues that might arise in a validated method.

METHOD TRANSFER FOR PROTEIN SEC CHARACTERIZATION

In 2010, Waters introduced ACQUITY UPLC Protein BEH SEC Columns containing 1.7 µm particles designed for optimal performance on Waters low dispersion, UPLC instrumentation. For the first time, these columns delivered outstanding component resolution in less time compared to use of traditional SEC column containing 5–8 µm particles. Waters XBridge Protein BEH SEC, 200Å and 450Å, 3.5 µm Columns can now effectively deliver comparable component resolution on HPLC platforms where high sample throughput is not a requirement.

Scalable Chromatography on Waters HPLC- vs. UPLC-based SEC Columns

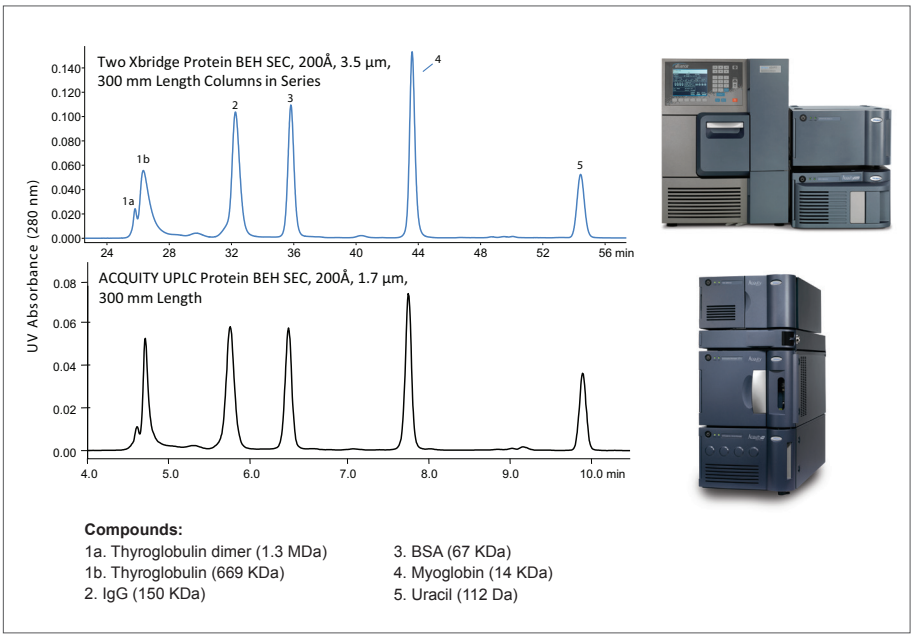


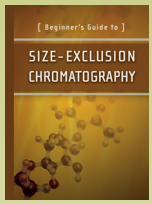
Figure 7. Separation of Waters BEH200 SEC Protein Standard (P/N 186006518) on two XBridge Protein BEH SEC 200Å, 3.5 µm, 7.8 x 300 mm Columns run in series using an Alliance® HPLC (top) and on a single ACQUITY UPLC Protein BEH SEC 200Å, 1.7 µm, 4.6 x 300 mm Column using an ACQUITY H-Class Bio UPLC (bottom). The flow rates were scaled based on particle diameter and column ID to 0.42 mL/minute for the two HPLC columns run in series and 0.3 mL/minute for the UPLC Column. Sample loads were also adjusted for column volume. Peak identities for chromatograms are: 1a) thyroglobulin dimer (1.3 MDa), 1b) thyroglobulin (669 KDa), 2) IgG (150 KDa), 3) BSA (67 KDa), 4) myoglobin (14 KDa), and uracil (112 Da).

A Review: Size-Exclusion Chromatography for the Analysis of Protein Biotherapeutics and Their Aggregates

The 23-page, Journal of Liquid Chromatography Publication provides an excellent historical review and recent advancements involving the use of SEC for the analysis of proteins. It discusses various instrumentation considerations as well as method development strategies for the successful use of this important bioanalytical technique.

Literature Code:
720004595EN

Beginner's Guide to Size-Exclusion Chromatography



This 62-page paperback book details the principles and practice of using size-based separations for polymer characterization. It provides readers a straightforward introduction to traditional gel permeation chromatography (GPC) and includes clear and colorful diagrams to acquaint the reader with basic SEC concepts including instrument and detection considerations.

Literature Code:
715004398

ORDERING INFORMATION

Description	Configuration	Particle Size	Dimension	Part No.
XBridge Protein BEH SEC, 200Å Column with BEH200 SEC Protein Standard Mix	Guard Column	3.5 µm	7.8 x 30 mm	176003594
XBridge Protein BEH SEC, 200Å Column with BEH200 SEC Protein Standard Mix	Column	3.5 µm	7.8 x 150 mm	176003595
XBridge Protein BEH SEC, 200Å Column with BEH200 SEC Protein Standard Mix	Column	3.5 µm	7.8 x 300 mm	176003596
XBridge Protein BEH SEC, 450Å Column with BEH450 SEC Protein Standard Mix	Guard Column	3.5 µm	7.8 x 30 mm	176003597
XBridge Protein BEH SEC, 450Å Column with BEH450 SEC Protein Standard Mix	Column	3.5 µm	7.8 x 150 mm	176003598
XBridge Protein BEH SEC, 450Å Column with BEH450 SEC Protein Standard Mix	Column	3.5 µm	7.8 x 300 mm	176003599
Straight Connection Tubing and Fittings for XBridge Protein BEH SEC Column	—	—	—	WAT022681
U-Bend Connection Tubing and Fittings for XBridge Protein BEH SEC Column	—	—	—	WAT084080
BEH 200Å SEC Protein Standard Mix	—	—	—	186006518
BEH 450Å SEC Protein Standard Mix	—	—	—	186006842

ADDITIONAL INFORMATION

Description	Literature Code
Alliance System Brochure	720000370EN
A Review: Size-Exclusion Chromatography for the Analysis of Protein Biotherapeutics and Their Aggregates	720004595EN
Beginner's Guide to Size-Exclusion Chromatography	715004398

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