

ACQUITY UPLC BEH125,  
BEH200 AND BEH450  
SEC PROTEIN STANDARDS

Size-exclusion chromatography continues to be a standard technique for the analysis of monoclonal antibodies and their aggregates.

The Ethylene-Bridged Hydrib (BEH) SEC column line is designed to provide a stable chemistry with minimal non-desired secondary interactions from proteins and peptides. Each batch of Protein Separation Technology SEC packaging material is tested and qualified using an appropriate test mixture. The same test mixtures are recommended to be used for functional benchmarking tests of a new column as well as for on going monitoring of system and column lifetime performance once that benchmark is established.

- The BEH125 SEC, 1.7  $\mu\text{m}$  column is designed for analysis of peptides and proteins in the molecular weight range of 1,000-80,000 daltons
- The BEH125 SEC Protein Standard Mix provides a suitable mixture of proteins within the qualified range.
- The ACQUITY UPLC® BEH200 SEC, 1.7  $\mu\text{m}$  column is designed to characterize proteins ranging in molecular weight from 10,000-450,000 daltons, including monoclonal IgG monomers and their aggregates
- The BEH200 SEC Protein Standard Mix provides a suitable mixture of proteins and an IgG to be able to provide a qualified test mix for this column.
- The BEH450 SEC, 2.5  $\mu\text{m}$  column is designed for analysis of peptides and proteins in the molecular weight range of 100,000 to 1,500,000 Daltons
- The BEH450 Protein Standard Mix provides a suitable mix of proteins within this range.

BEH125 SEC Test Mix

Analyte	pI	MW
1. Thyroglobulin, 0.1 mg/mL	4.6	669,000
2. Ovalbumin, 0.3 mg/mL	4.5	44,200
3. Ribonuclease A, 0.3 mg/mL	9.6	13,700
4. Uracil, 0.05 mg/mL	N/A	112

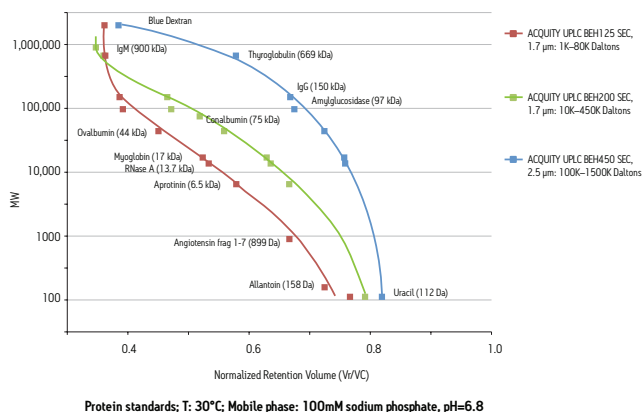
BEH200 SEC Test Mix

Analyte	pI	MW
1. Thyroglobulin, 3 mg/mL	4.6	669,000
2. IgG, 2 mg/mL	6.7	150,000
3. BSA, 5 mg/mL	4.6	66,400
4. Myoglobin, 2 mg/mL	6.8, 7.2	17,000
5. Uracil, 0.1 mg/mL	N/A	112

BEH450 SEC Test Mix

Analyte	pI	MW
1. Thyroglobulin Dimer	4.6	1.4 million
2. Thyroglobulin, Approx 3 mg/mL	4.6	669,000
3. IgG, 2 mg/mL	6.7	150,000
4. BSA, 5 mg/mL	4.6	66,400
5. Myoglobin, 2 mg/mL	6.8, 7.2	17,000
6. Uracil, 0.1 mg/mL	N/A	112

Figure 1. Calibration Curves for ACQUITY UPLC BEH125, BEH200 and BEH450, SEC Columns (Note: Bolded are Proteins within each standard)



## Performance Testing

It is recommended to perform an efficiency test on every column before using it. Waters recommends using a suitable solute mixture, as found in both the SEC Protein Standard mixes, to analyze the column upon receipt, determine the number of theoretical plate counts (N) for periodic comparisons and for repeat testing at predetermined intervals to track column health and performance over time as well as determining differences in systems due to quality connections, operating environment, system electronics, reagent quality, column condition and operator technique.

- The standards come ready-to-use. It is in a lyophilized powder for stability and can be directly solubilized with 500 µl, 100 mM Sodium Phosphate Buffer, pH 6.8 (See Care and Use Manual P/N 720003385 for information on buffer preparation).
- The standard was specifically designed with quality control criteria to contain, relevant and meaningful data that our manufacturing plant in Taunton uses to QC the respective columns.

## Example of Performance Test Results Using the appropriate SEC Protein Mixes

Figure 2. Protein mixture separation on an ACQUITY UPLC BEH125 SEC, 1.7 µm column.

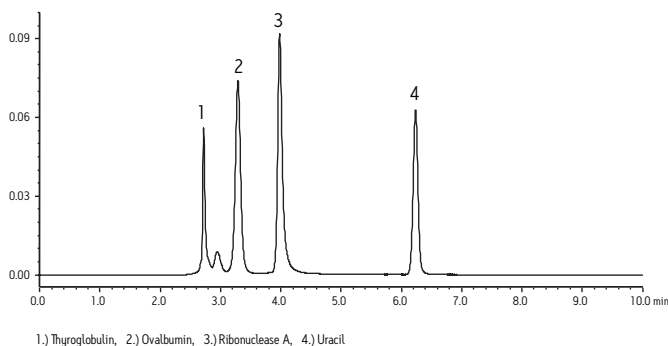


Figure 3. Protein mixture separation on an ACQUITY UPLC BEH200 SEC, 1.7 µm column.

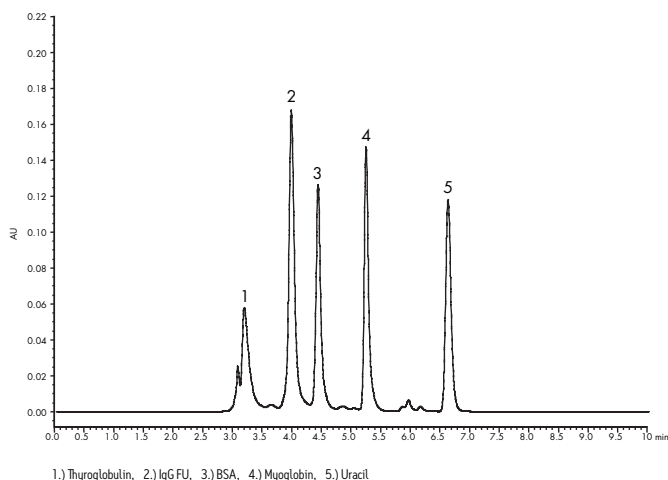
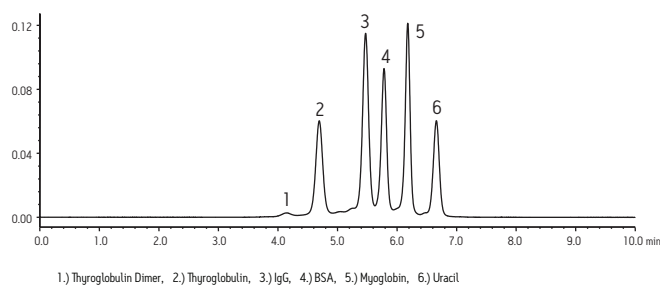


Figure 4. Protein Mixture on an ACQUITY UPLC BEH450 SEC, 2.5 µm column.



## Examples of Lot Reproducibility

Each lot of the SEC Protein Mix is manufactured under strict quality control criteria. Each lot is monitored and compared to previous lots. Any changes in raw material are noted on the Certificate of Analysis. In addition, there is superb reproducibility within each lot from vial to vial.

Figure 5. ACQUITY UPLC BEH200 SEC Protein Standard Mix: Reproducibility between Samples in the Same Lot.

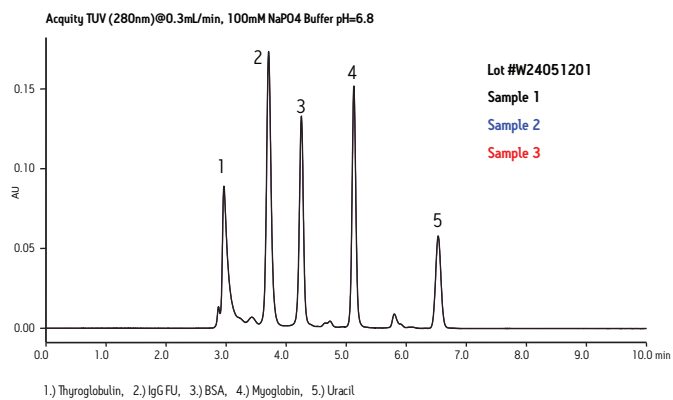


Figure 6. ACQUITY UPLC BEH125 SEC Protein Standard Mix: Reproducibility between Samples in the Same Lot

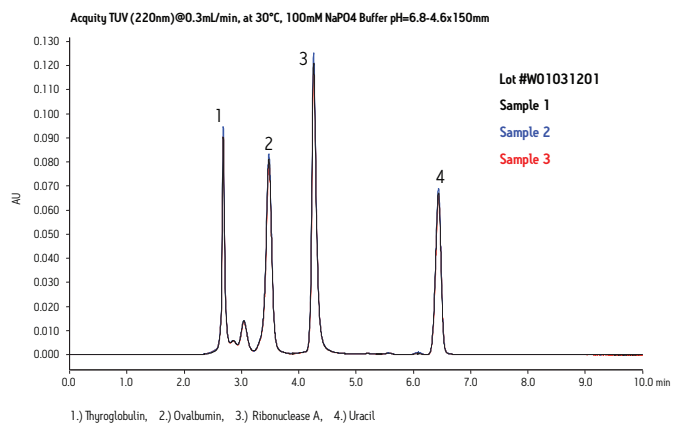


Figure 7. ACQUITY UPLC BEH200 SEC Protein Standard Mix: 3 different lots and their reproducibility

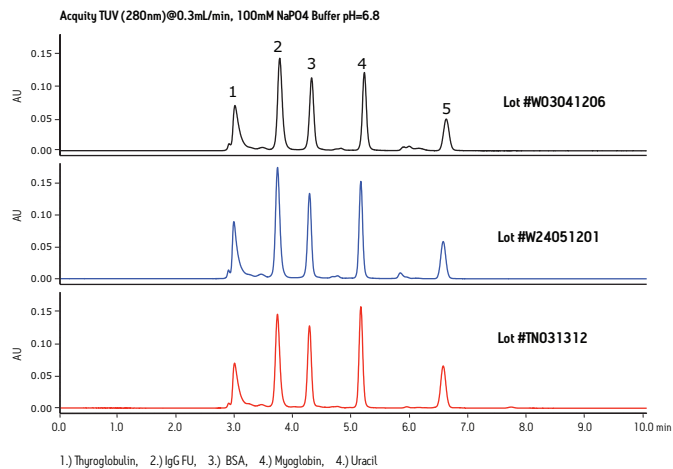
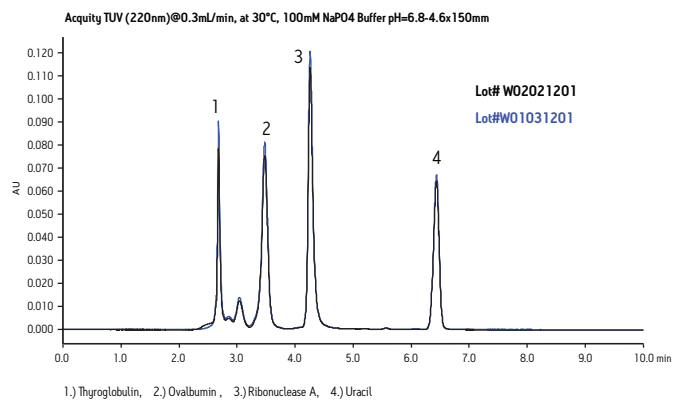


Figure 8. ACQUITY UPLC BEH125 SEC Protein Standard Mix: 2 different lots and their reproducibility



## ORDERING INFORMATION

Description	Particle Size	Dimensions	Part No.
BEH125 SEC Protein Standard Mix			186006519
BEH200 SEC Protein Standard Mix			186006518
BEH450 SEC Protein Standard Mix			186006842
ACQUITY UPLC BEH125 SEC column	1.7 µm	4.6 x 150 mm	186006505
ACQUITY UPLC BEH125 SEC column	1.7 µm	4.6 x 300 mm	186006506
ACQUITY UPLC BEH125 SEC guard column kit	1.7 µm	4.6 x 30 mm	186006504
ACQUITY UPLC BEH200 SEC column	1.7 µm	4.6 x 150 mm	186005225
ACQUITY UPLC BEH200 SEC column	1.7 µm	4.6 x 300 mm	186005226
ACQUITY UPLC BEH200 SEC guard column kit	1.7 µm	4.6 x 30 mm	186005793
ACQUITY UPLC BEH450 SEC column	2.5 µm	4.6 x 150 mm	186006851
ACQUITY UPLC BEH450 SEC column	2.5 µm	4.6 x 300 mm	186006852
ACQUITY UPLC BEH450 SEC guard column kit	2.5 µm	4.6 x 30 mm	186006850
ELSD outlet tubing (0.004" id x 6" length)			430001562
0.005 x 1.75" SEC UPLC Connection Tubing, 2/pk			186006613

Notes: Size-exclusion chromatography may require modifications to an existing ACQUITY UPLC system. Please refer to "Size-Exclusion and Ion-Exchange Chromatography of Proteins using the ACQUITY UPLC System", (715002147) or "Size-Exclusion and Ion-Exchange Chromatography of Proteins using the ACQUITY UPLC H-Class System", (715002909) for specific recommendations.

Austria and European Export (Central South Eastern Europe, CIS and Middle East) 43 1 877 18 07, Australia 61 2 9933 1777, Belgium 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 and India Subcontinent 91 80 2837 1900, Ireland 353 1 448 1500, Italy 39 02 265 0983, Japan 81 3 3471 7191, Korea 82 2 6300 4800, Mexico 52 55 52 00 1860, The Netherlands 31 76 508 7200, Norway 47 6 384 6050, Poland 48 22 101 5900, 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, United Kingdom 44 208 238 6100, All other countries: Waters Corporation U.S.A. 1 508 478 2000/1 800 252 4752

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