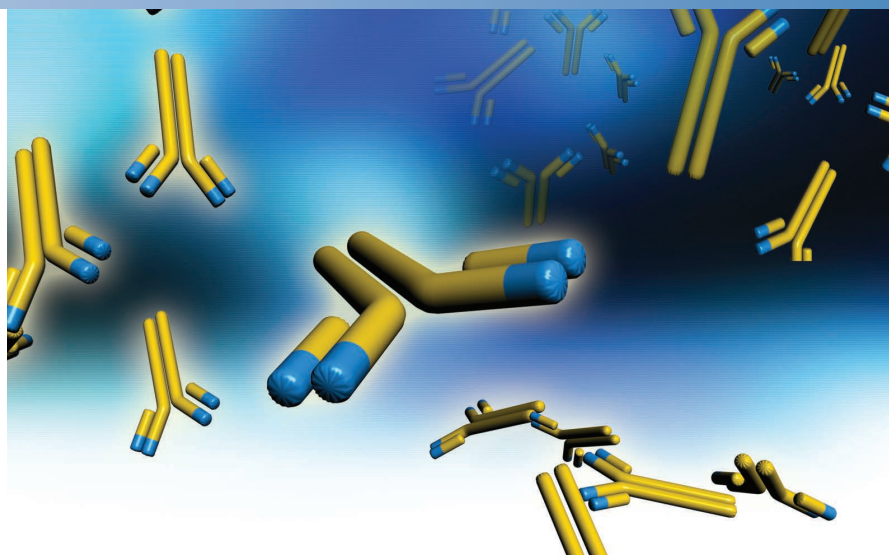
MAbPac SEC-1 Column

High Performance Size-Exclusion Chromatography Column for Monoclonal Antibody Analysis

The Thermo Scientific™ MAbPac™ SEC-1 is a size-exclusion chromatography (SEC) column designed for mAb characterization, including the separation of monomers, aggregates, and fragments. The unique chemistry is stable under both denaturing and non-denaturing conditions, using high-salt, low-salt, or volatile mobile phases.

Product Highlights

- Proprietary hydrophilic bonded layer results in minimal non-desired interactions between the proteins and the stationary phase.
- Stable surface bonding leads to low column bleed and compatibility with MS, ELSD and Thermo Scientific™ Dionex™ Corona™ Aerosol Detection.
- Rugged, reproducible column packing.
- Superior performance for the analysis of monoclonal antibodies, aggregates, and their fragments.



Introduction

Monoclonal antibodies (mAbs) are a growing family of therapeutic proteins. For final biopharmaceutical product approval and subsequent manufacturing, a comprehensive characterization of mAb purity, aggregate forms and charge variants is required by regulatory agencies. mAbs produced from mammalian cell culture may contain significant amounts of dimers and higher-order aggregates. Size exclusion chromatography (SEC) is a well-accepted technique for the detection and accurate quantification of protein aggregates in biological drug products. It is also routinely used for the characterization and quality control of mAb products.

Column Technology

The MAbPac SEC-1 column is based on high-purity, spherical, porous (300 Å), 5 µm silica particles covalently modified with a proprietary diol hydrophilic layer. The column is packed into three different ID formats (7.8, 4.0, and 2.1 mm) to accommodate different applications and sample loadings. The 7.8 mm ID column provides the highest resolution of mAb and aggregates separation and rugged method for routine analysis. The 4.0 mm ID column offers excellent resolution at greatly reduced mobile phase consumption and requires modern HPLC system with minimal extra column volumes. The 2.1 mm ID column provides the highest sensitivity for small amount of sample loading, and is suitable for direct MS detection.

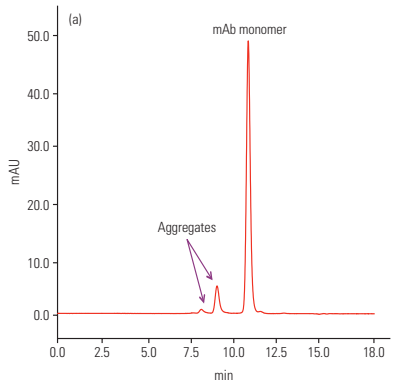
Applications

Analysis of mAb and Aggregates

mAbs produced from mammalian cell culture may contain significant amounts of dimers, trimers and other higher order aggregates. The formation of aggregates may originate from elevated temperature, shear strain, surface adsorption, high protein concentration or other unknown reasons. Studies show that the aggregates present in drug products can cause severe immunogenic and anaphylactic reactions. Thus, biopharmaceutical manufacturers are required to develop analytical methods to characterize the biopharmaceuticals and monitor the efficacy and safety as per the guidelines of the FDA and other regulatory agencies. The MAbPac SEC-1 is specially designed for analysis of mAbs and their aggregates (Figure 1a, 1b and 1c). Among the three I.D. column formats, the 7.8 mm I.D. column has the highest resolution and baseline separates mAb, its dimer, and its trimer. The 4.0 mm I.D. column has lower resolution comparing to the 7.8 mm I.D. column but nevertheless can still baseline separate mAb and its dimer. The 2.1 mm I.D. column has the lowest resolution. However, the 2.1 mm I.D. column has the highest sensitivity and consumes the least amount of sample. The flow rates applied to the 4.0mm I.D. column and the 2.1 mm I.D. column are 200 µL/min and 50 µL/min respectively. Both conditions are compatible with direct MS detection.

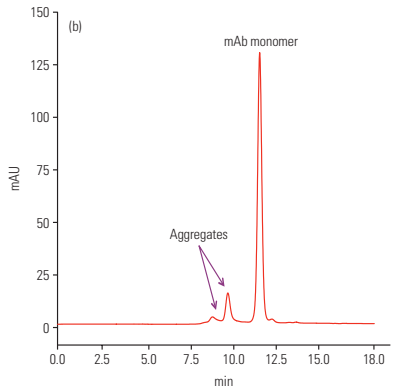
Analysis of mAb Fragments

Full characterization of mAb includes determination of mass of the mAb fragments, such as heavy chain (HC) and light chain (LC) generated by reduction of inter chain disulfide bonds, as well as Fab and Fc generated by papain digestion. Using denaturing mobile phase containing 20% acetonitrile, 0.1% TFA, and 0.05% formic acid, SEC enables analysis of mAb (Figure 2a), baseline separation of HC and LC (Figure 2b), as well as partial separation of Fab and Fc (Figure 2c). It serves as a platform method for mAb fragment analysis. In addition, this mobile phase is compatible with direct mass spectrometry detection.



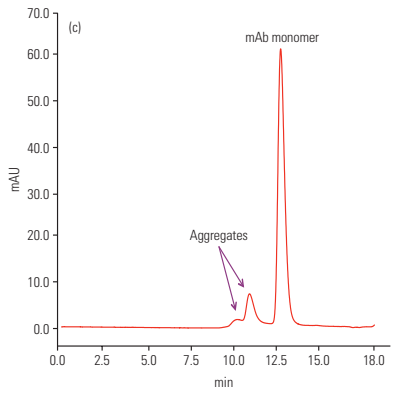
Column:	**MAbPac SEC-1**, 5 µm,
Dimension:	7.8 × 300 mm
Mobile Phase:	50 mM sodium phosphate pH 6.8, in 300 mM sodium chloride
Flow Rate:	760 µL/min
Inj. Volume:	10 µL
Temp.:	30 °C
Detection:	280 nm
Sample:	mAb (1 mg/mL)

Figure 1a: Analysis of monoclonal antibody (mAb) and aggregates (7.8 × 300 mm)



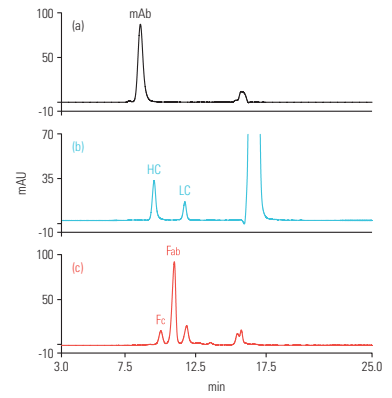
Column:	**MAbPac SEC-1**, 5 µm,
Dimension:	4.0 × 300 mm
Mobile Phase:	50 mM sodium phosphate pH 6.8, in 300 mM sodium chloride
Flow Rate:	200 µL/min
Inj. Volume:	5 µL
Temp.:	30 °C
Detection:	280 nm
Sample:	mAb (1 mg/mL)

Figure 1b: Analysis of monoclonal antibody (mAb) and aggregates (4.0 × 300 mm)



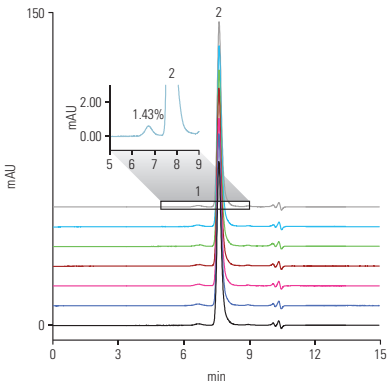
Column:	**MAbPac SEC-1**, 5 µm,
Dimension:	2.1 × 300 mm
Mobile Phase:	50 mM sodium phosphate pH 6.8, in 300 mM sodium chloride
Flow Rate:	50 µL/min
Inj. Volume:	1 µL
Temp.:	30 °C
Detection:	280 nm
Sample:	mAb (1 mg/mL)

Figure 1c: Analysis of monoclonal antibody (mAb) and aggregates (2.1 × 300 mm)



Column:	**MAbPac SEC-1**, 5 µm,
Dimension:	4.0 × 300 mm
Mobile Phase:	20% acetonitrile, 0.1% formic acid, 0.05% trifluoroacetic acid
Flow Rate:	200 µL/min
Inj. Volume:	5 µL
Temp.:	30 °C
Detection:	280 nm
Samples:	(a) mAb (b) mAb reduction by DTT (c) mAb digestion by papain

Figure 2: mAb and mAb fragments analysis using denaturing mobile phase

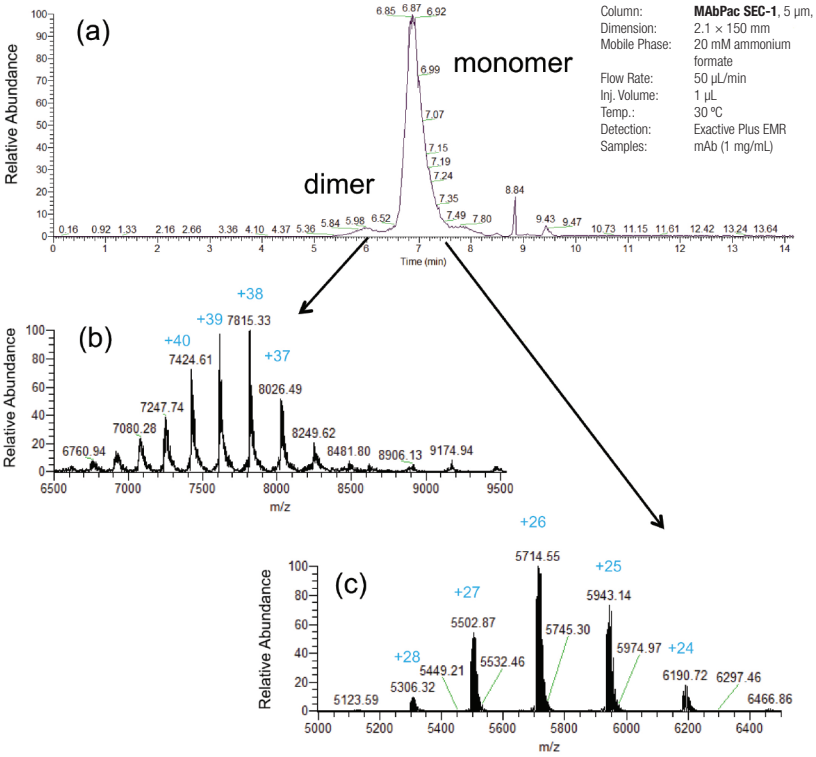


Column: **MAbPac SEC-1**, 5 µm,
Dimension: 4.0 × 300 mm
Mobile Phase: 50mM sodium phosphate pH 6.8, in 300 mM sodium chloride
Flow Rate: 300 µL/min
Inj. Volume: 5 µL
Temp.: 30 °C
Detection: 280 nm
Sample: mAb (1 mg/mL)

Peaks: 1. mAb dimer
2. mAb monomer

Injection #	Monomer Retention Time	Asymetry (10%)	Efficiency (Plates)	Dimer Retention Time	Pressure (psi)
10	7.71	1.39	7287	6.75	1017
100	7.71	1.36	7333	6.75	1020
160	7.71	1.37	7310	6.75	1020
250	7.71	1.35	7321	6.75	1027
319	7.71	1.33	7311	6.75	1023
467	7.71	1.35	7357	6.75	1027
521	7.71	1.34	7357	6.75	1027

Figure 3: Excellent ruggedness for mAb analysis



Column: **MAbPac SEC-1**, 5 µm,
Dimension: 2.1 × 150 mm
Mobile Phase: 20 mM ammonium formate
Flow Rate: 50 µL/min
Inj. Volume: 1 µL
Temp.: 30 °C
Detection: Exactive Plus EMR
Samples: mAb (1 mg/mL)

Figure 4: SEC-MS analysis of MAb dimer aggregates and monomer under non-denaturing condition

Reproducibility

Rugged column packing is a critical characteristic for accurate and reproducible results, as well as good column lifetime. MAbPac SEC-1 columns are packed using a carefully developed packing protocol to ensure excellent packed bed stability, column efficiency and peak asymmetry. Figure 3 and the corresponding data in Table 1 demonstrate that even after 500 cycles of operation with intermittent injections of a mAb sample, the MAbPac SEC-1 column still maintains excellent performance, providing consistent retention time, peak shape, and peak efficiency, with minimal increase in column backpressure. The area of the dimer peak was calculated and the percent of the dimer was shown as an inset relative to the main peak.

SEC-MS

The analysis of mAbs by SEC is typically performed under non-denaturing conditions at near-physiological pH range (6.8). The commonly used buffer is phosphate buffer with 300 mM NaCl. However, the non-volatile nature of phosphate buffer and high salt content makes this buffer non-compatible with online mass spectrometry detection. Using volatile buffer such as 20 mM ammonium formate, MAbPac SEC-1 can be directly coupled to a high resolution mass spectrometer for MS detection. Separation of mAb dimer aggregate and monomer is achieved on a short SEC column (2.1 × 150 mm) within 8 min (Figure 4a). Both dimer aggregate and monomer are successfully detected (Figure 4b and 4c). Charge states are labeled in blue.

Physical Data

Bonding chemistry	Diol
Silica substrate	Spherical, high-purity porous silica
Particle size	5 µm
Pore size	300 Å
Column housing	PEEK for 4.0 mm I.D. columns SST for 7.8 mm and 2.1 mm I.D. columns
Separation range for globular proteins	10,000–1,000,000
Exclusion limit for globular proteins	>1,000,000

Operational Specifications

Dimension (mm)	Dimension (mm)	Flow Rate (µL/min)	Pressure Limit (psi)	Temperature (°C)	pH Range
MAbPac SEC-1	7.8 × 300	760–1,000	< 1,000	< 30	2.5–7.5
MAbPac SEC-1	4.0 × 300	200–300	< 1,000	< 30	2.5–7.5
MAbPac SEC-1	4.0 × 150	200–300	< 600	< 30	2.5–7.5
MAbPac SEC-1	4.0 × 50	200–300	< 200	< 30	2.5–7.5
MAbPac SEC-1	2.1 × 300	50–75	< 1,000	< 30	2.5–7.5
MAbPac SEC-1	2.1 × 150	50–75	< 600	< 30	2.5–7.5

Ordering Information

Description	Particle Size	Part Number
MAbPac SEC-1, Analytical, 7.8 × 300 mm	5 µm	088460
MAbPac SEC-1, Analytical, 4 × 300 mm	5 µm	074696
MAbPac SEC-1, Analytical, 4 × 150 mm	5 µm	075592
MAbPac SEC-1, Guard, 4 × 50 mm	5 µm	074697
MAbPac SEC-1, Analytical, 2.1 × 300 mm	5 µm	SP6937
MAbPac SEC-1, Analytical, 2.1 × 150 mm	5 µm	SP6938

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