



HPLC columns

MAbPac SEC-1 columns

Product manual

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Introduction

Introduction to the MAbPac SEC-1 column

The Thermo Scientific™ MAbPac™ SEC-1 Column is a size exclusion chromatography (SEC) column specifically designed for separation and characterization of monoclonal antibodies (mAbs). The stationary phase is designed to handle different eluent conditions containing both high and low ionic strength mobile phases, as well as mass spectrometry friendly volatile eluents. SEC is a common chromatographic technique for separating biomolecules based on their sizes. On the SEC column, very large analytes (>1000 kDa) are excluded by the pores thus eluting in the void, whereas smaller analytes (<1000 Da) pass through the pores and elute according to their sizes – larger analytes elute earlier than smaller analytes.

The MAbPac SEC-1 is based on high-purity, spherical, porous (300 Å), 5 µm silica particles that are covalently modified with a proprietary diol hydrophilic layer. This proprietary process brings an extremely low level of non-desired interaction sites. The 7.8 mm and 2.1 mm small internal diameter (I.D.) columns are packed into stainless steel housing while the 4.0 mm I.D. format columns are packed into PEEK housing.

MAbPac SEC-1 operating limits and specifications

Table 1. Operating conditions

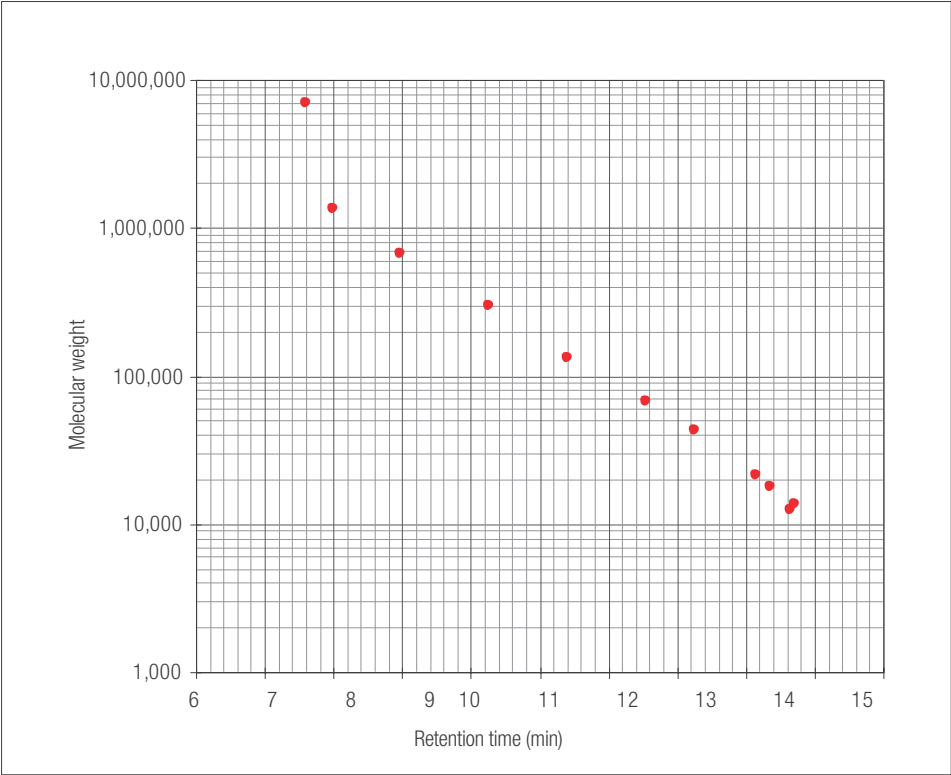
Parameter	Recommendation
Flow rate range (recommended)	760 – 1,000 µL/min for the 7.8 mm I.D. columns 200 – 300 µL/min for the 4.0 mm I.D. columns 50 – 75 µL/min for the 2.1 mm I.D. columns
Shipping solution/ long term storage solution	20% acetonitrile in deionized water
Typical buffers	Phosphate buffer with NaCl, e.g. 50 mM phosphate buffer (pH 6.8) + 0.3 M NaCl Good's buffer with NaCl, e.g. 20 mM MES buffer (pH 6.1) + 0.3 M NaCl Ammonium formate or ammonium acetate solutions, pH 5 – 7
Solvents compatibility	Compatible with 100% organic solvents
Detergent compatibility	Compatible with 0.1% SDS, though binding will be irreversible and it is recommended that the column be dedicated to this application
Temperature range	20 – 30 °C
Pressure limit	1,000 psi for 300-mm long columns 600 psi for 150-mm long columns
pH range	2.5 – 7.5

Table 2. Physical characteristics

Parameter	Recommendation
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 Dalton
Exclusion limit for globular proteins	>1,000,000

Calibration curve

The MAbPac SEC-1 column has a wide molecular operating range, as shown in the molecular weight calibration curve.



MAbPac SEC-1 column, 4 × 300 mm	
Cat. no	074696
Mobile phase	50 mM sodium phosphate buffer (ph 6.8) + 0.3M NaCl
Flow rate	0.20 mL/min
Inj. volume	5 µL
Temperature	25 °C
Detection	UV, 220 nm
Analytes	1. Thyroglobulin
	2. γ-Globulin
	3. BSA
	4. Ovalbumin
	5. Trypsin inhibitor
	6. Myoglobin
	7. Ribonuclease A
	8. CytochromeC

Protein	Retention time (min)	MW (kDa)
γ-Globulin aggregate	7.6	7,000*
Thyroglobulin dimer	8.0	1,338
Thyroglobulin	9.0	669
γ-Globulin dimer	10.3	300
BSA dimer	11.4	134
BSA	12.6	67
Ovalbumin	13.3	43
Trypsin inhibitor	14.1	22
Myoglobin	14.4	18
Ribonuclease A	14.7	14
Cytochrome C	14.6	12

*Tentative, not actual

Getting started:

Step-by-step procedure

Thermo Fisher Scientific recommends that you perform an efficiency test on your MAbPac SEC-1 column before use. The purpose of column performance validation is to ensure no damage has occurred during shipping. Steps 1 – 5 below outline the necessary steps to perform this validation test. Test the column using the conditions described on the Quality Assurance (QA) report enclosed in the column box. Repeat the test periodically to track the column performance over time. Note that slight variations may be found on two different HPLC systems due to system electronic, hardware, plumbing, operating environment, reagent quality, column conditioning, and operator technique.

Visually inspect the column

Report any visible damage upon receiving the column to Thermo Fisher Scientific immediately. Depending upon the nature of the damage, we may request that you return the damaged column back to us for a replacement column.

Mobile phase selection

The MAbPac SEC-1 column can be used with a variety of mobile phases. The columns are normally used with 20 – 100 mM buffer (pH 5.0 – 7.5) containing 0.1 – 0.3 M salt. A typical mobile phase for protein separations is 50 mM phosphate buffer (pH 6.8) + 0.3 M NaCl. Ammonium formate and ammonium acetate buffer can also be used when coupling the column to a mass spectrometer and a volatile buffer is needed, in which case 100 mM buffer concentration is a good starting point. It is highly recommended that the mobile phase is pre-filtered with a 0.2 µm pore size membrane filter, or/and an in-line filter containing installed membrane on HPLC system.

Set up the LC system

Use a standard LC system equipped with a LC pump, a column oven, a UV detector (210 – 220 nm and/or 280 nm) and an injector (or an autosampler). It is required that the system be optimized for low dead volume; usage of I.D. tubing (especially between the injector and detector) and a proper detector flow cell is required for best results. See Table 3 for recommended chromatographic setup. The system should be thoroughly primed before use. It is recommended the column is run at room temperature (20 °C to 30 °C) to achieve better column lifetime.

Table 3. Recommended chromatographic setup

Column ID (mm)	2.1	4.0	7.8
Flow rate (µL/min)	50 – 75	200 – 300	760 – 1,000
UV flow cell	Micro (180 nL)	Semi-micro (2.5 µL)	Analytical (11 µL)
Tubing ID (µm)	50 – 75	75 – 100	150 – 250
Sample injection size (µL)	1 – 2	5 – 10	20 – 50

Operational guidelines

- Avoid any sudden pressure surge
- Operate within column specifications (see “Operating conditions”)
- Follow the direction of flow that is marked on the column
- Column conditioning is recommended upon initial column use: inject 4 successive injections of the sample, or 100 µg BSA, or other proteins of choice
- Reverse flow should be avoided except for removal of inlet blockage (see “Column care”)
- It is recommended that MAbPac SEC-1 columns be stored in 20% acetonitrile in deionized water. If stored in an aqueous buffer solution, made sure the pH of storage solution is between 4 and 6 and contain 0.05% sodium azide.

Getting started:

Step-by-step procedure (continued)

- Buffers are recommended during use of this column: typically a combined concentration of 20 mM (to avoid any possible undesirable interactions with the packing material).
- The use of a guard column is recommended when injecting dirty samples to protect the analytical column and to extend the column lifetime.
- If necessary, run several blanks between injections to minimize carry-over.
- Salt concentration should not exceed 0.5 M.
- Solvent compatibility: This column is compatible with 100% organic solvents (i.e., acetonitrile and methanol). However, take precautions not to precipitate any salts used in the mobile phase present on the column.
- Detergent compatibility: Compatible with 0.1% SDS, though binding will be irreversible and it is recommended that the column be dedicated to this application.

Reproduce the chromatogram in the lot validation report

Perform the column QA test using the conditions described in the QAR, and compare the result with the reported values.

The column should be fully equilibrated before any injection.

At least three injections should be made to assess the reproducibility. Once you are satisfied with the column performance report result, proceed to the next step.



Note

Due to various reasons, such as difference of LC systems, mobile phases, etc, you may observe somewhat different separation from that in the report.

Real sample analysis

Once the column performance is satisfactorily confirmed in "Reproduce the chromatogram in the lot validation report", the column is ready for real sample analysis.



Note

It is recommended that the column performance test be performed periodically to monitor the condition of the column. Please compare it to initial performance test to note any changes. If the results are comparable and you are satisfied with the column performance, continue to use the same column for your applications. If you are not satisfied please proceed to the column washing procedure (see "Column washing procedure").

Column care

Column storage

The column can be stored in the mobile phase for short-term storage. For long-term storage (more than 5 days), it is recommended to store the column in a solution containing 20% acetonitrile in deionized water.

Operating pH range: pH 2.5 to 7.5

The column lifetime is heavily affected by the pH of the buffer and other chromatographic conditions. To obtain better column lifetime, it is recommended to use mobile phases with pH between 5.0 and 7.0.

Operating temperature limit: up to 30 °C

Based on our experimental data, this column can be used at 30 °C. The typical operating temperature for most applications is in the range of 20 °C – 25 °C.

Pressure limit: 1,000 psi for 300 mm long column; 600 psi for 150 mm long column

It is extremely important not to impose a sudden column pressure surge. Although the column pressure is rated up to 1,000 psi for a 300 mm long column, the typical operating pressure at 300 µL/min should not exceed 800 psi.

Flow rate

Please refer to Table 3 for recommended flow rate. Please note that the analyte efficiency may be affected at a higher flow rate.

Injection volume

Please refer to Table 3 for recommended injection volume. Large injection volume may affect resolution.

Column washing procedure



Note

The following procedure is designed for the 4.0 mm I.D. column. Please adjust the flow rate for different I.D. columns (760 µL/min for 7.8 mm I.D., or 50 µL/min for 2.1 mm I.D.).

Particulates in the sample or the mobile phase will plug the column inlet frit. If solvent flow appears to be restricted (high column back-pressure), check first to see that solvent flow is unobstructed up to the column inlet. If the column has the restriction, there may be particulate matter on the inlet frit. An attempt should be made to remove any inlet debris by back-flushing 25 to 30 mL of mobile phase through the column (at 200 µL/min). If this fails to return the column to near its original operating pressure, consider replacing the column.

Make sure that the mobile phases and samples are free of such particulates by filtering the eluents and samples with a 0.2 µm filter prior to use. In the event that column washing/cleaning is needed, the following procedure can be used as a guideline:

1. Wash the column with 100% D.I. water at 200 µL/min for 30 minutes.
2. Wash the column with 80% methanol at 200 µL/min for 30 minutes.
3. Wash the column with 0.1 M ammonium acetate pH 5.0 buffer at 200 µL/min for 30 minutes.
4. Wash the column with the mobile phase at 200 µL/min for at least 30 minutes.

Example applications

Separation of mAbs and their aggregates

Size-exclusion chromatography (SEC) is a well-accepted technique for the detection and accurate quantification of protein aggregates in biological drug products. The MAbPac SEC-1 column is specially designed for analysis of mAbs and their aggregates (Figure 1a, 1b and 1c). When mAbs are produced from mammalian cell culture, it 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.

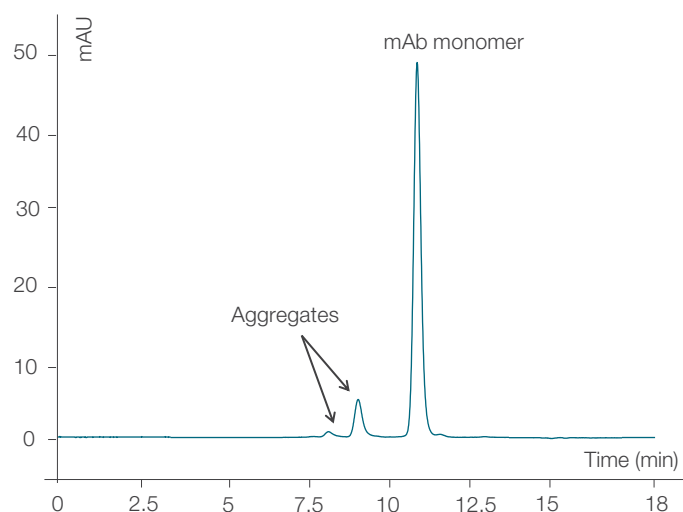


Figure 1a. Analysis of mAb and aggregates (7.8 × 300 mm)

MAbPac SEC-1 column, 5 µm, 7.8 × 300 mm	
Cat. no	088460
Mobile phase	50 mM sodium phosphate pH 6.8, in 300 mM NaCl
Flow rate	760 µL/min
Inj. volume	10 µL
Temperature	30 °C
Detection	280 nm
Sample:	mAb (1 mg/mL)

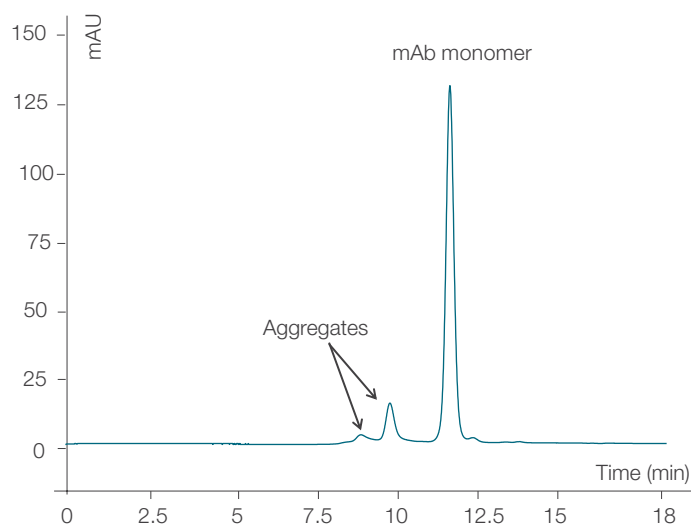
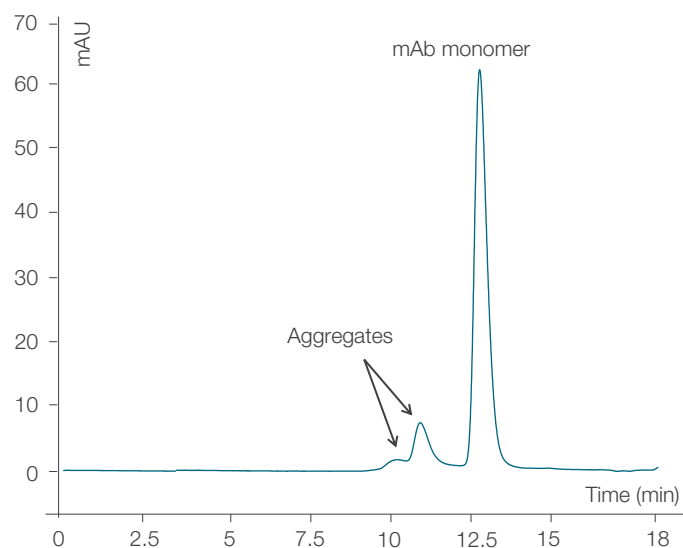


Figure 1b. Analysis of mAb and aggregates (4.0 × 300 mm)

MAbPac SEC-1 column, 5 µm, 4.0 × 300 mm	
Cat. no	074696
Mobile phase	50 mM sodium phosphate pH 6.8, in 300 mM NaCl
Flow rate	200 µL/min
Inj. volume	5 µL
Temperature	30 °C
Detection	280 nm
Sample:	mAb (1 mg/mL)

Example applications (continued)



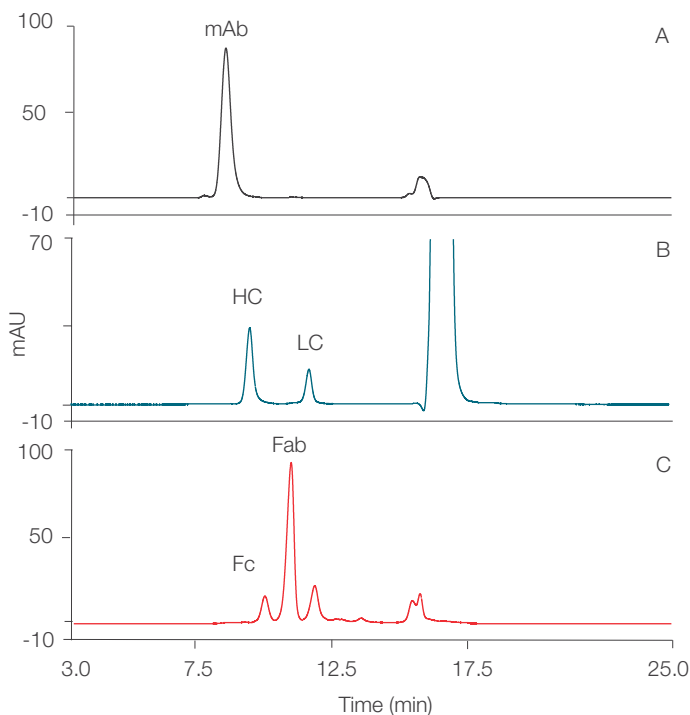
MAbPac SEC-1 column, 5 μm, 2.1 $\times$ 300 mm	
Cat. no	088789
Mobile phase	50 mM sodium phosphate pH 6.8, in 300 mM NaCl
Flow rate	50 μ L/min
Inj. volume	1 μ L
Temperature	30 $^{\circ}$ C
Detection	280 nm
Sample:	mAb (1 mg/mL)

Figure 1c. Analysis of mAb and aggregates (2.1 $\times$ 300 mm)

Separation 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 eluent 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 eluent is compatible with direct mass spectrometry detection.

Example applications (continued)

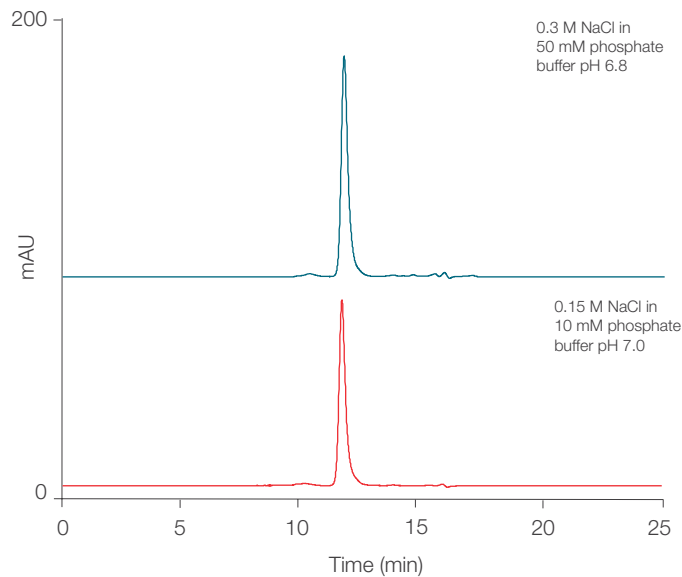


MABPac SEC-1 column, 5 µm, 4.0 x 600 mm	
Cat. no	074696
Mobile phase	20% acetonitrile, 0.1% FA, 0.05% TFA
Flow rate	200 µL/min
Inj. volume	5 µL
Temperature	30 °C
Detection	280 nm
Sample:	A: mAb B: mAb reduction by DTT C: mAb digestion by papain

Figure 2. mAb and mAb fragments analysis using denaturing eluent

Separation of mAbs using high and low ionic strength eluents

The MABPac SEC-1 column utilizes a diol hydrophilic layer prepared by a proprietary process and results in extremely low level of non-desired interaction sites. Combined with the use of the non-metal and bio-compatible PEEK column housing, it is



ideal for separating monoclonal antibodies, including monomer, aggregates and mAb fragments, by providing excellent peak shapes and efficiency for mAbs under both high and low salt conditions. As shown in Figure 3, separation of mAb with MABPac SEC-1 under both high salt condition (0.3 M NaCl in 50 mM phosphate buffer, upper C-gram) and low salt condition (0.15 M NaCl in 10 mM phosphate buffer, lower C-gram), displayed good peak shape and peak efficiencies.

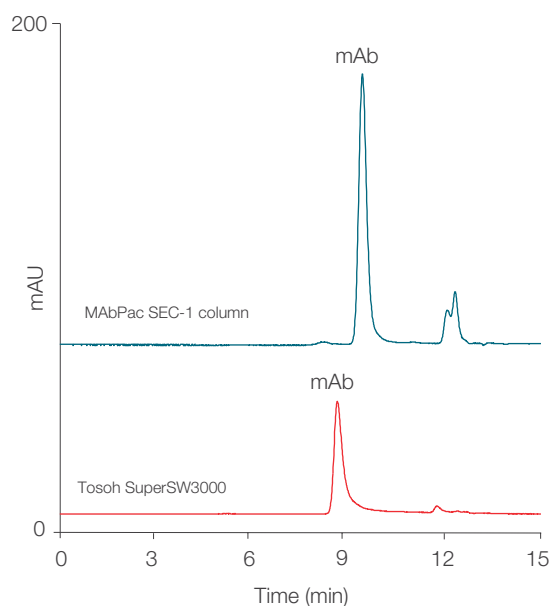
MABPac SEC-1 column, 5 µm , 4.0 x 300 mm	
Cat. no	074696
Mobile phase	0.3 M NaCl in 50 mM phosphate buffer pH 6.8 (upper C-gram); 0.15 M NaCl in 10 mM phosphate buffer pH 7.0 (lower C-gram);
Flow rate	200 µL/min
Inj. volume	5 µL
Temperature	30 °C
Detection	280 nm
Sample:	mAb (1 mg/mL)

Figure 3. mAb analysis using MABPac SEC-1 in high-salt and low-salt eluents

Example applications (continued)

Separation of mAbs using volatile buffers

The proprietary bonding chemistry of the MAbPac SEC-1 column produces a hydrolytically stable hydrophilic bonded layer and extremely low column bleed, making it fully compatible with MS, Corona CAD or ELSD detection. Figure 4 shows the analysis of a mAb in 100 mM ammonium acetate buffer, a MS-compatible mobile phase, on a MAbPac SEC-1 (PEEK) column. The MAbPac SEC-1 (PEEK) mAb separation exhibited high efficiency, good peak shape and recovery under these conditions making it useful for online MS analysis



MAbPac SEC-1 column, 5 µm Tosoh SuperSW3000 column	
Dimension	4.0 × 300 mm
Mobile phase	0.1 M NH ₄ OAc, pH5
Flow rate	250 µL/min for MAbPac SEC-1
Inj. volume	2.0 µL on MAbPac SEC-1 2.5 µL on Tosoh SuperSW3000
Temperature	25 °C
Detection	280 nm
Sample:	mAb (1 mg/mL in buffer)

Note: Flow rate and injection volume are adjusted for the same linear velocity and relative loading to make fair comparison.

	MAbPac SEC-1 column	Tosoh SuperSW3000
PW (50% height)	0.256	0.296 min
Efficiency (plates)	6780	005
Asymmetry	1.31	2.14
Peak height (mAU)	105.3	43.7

Figure 4. mAb analysis in volatile buffer MAbPac SEC-1 column vs. Tosoh™ TSKgel™ SuperSW3000 column

Example applications (continued)

Ruggedness of MAbPac SEC-1 column

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 5 demonstrates that even after 500 runs with intermittent injections of MAb samples, the MAbPac SEC-1 column still maintained excellent performance, consistent retention time, peak shape and peak efficiency, with a stable column backpressure. The area of the dimer peak was calculated and the percent of dimer was shown as an inset relative to the main peak.

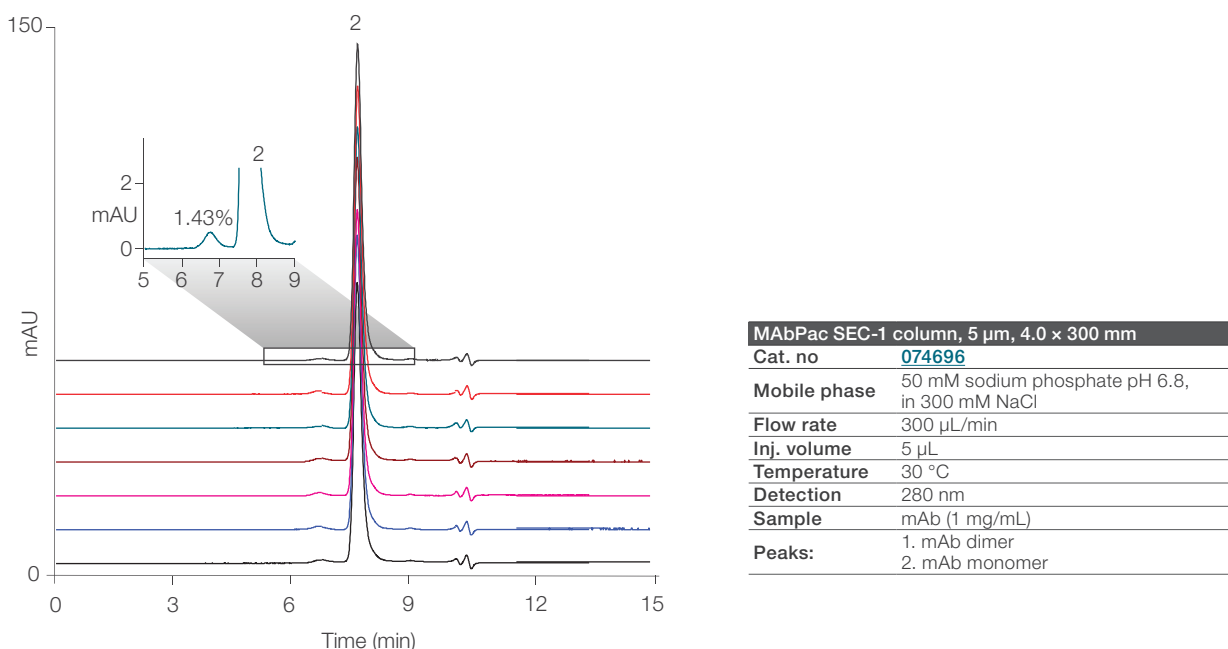


Figure 5. Excellent ruggedness for mAb analysis

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	7257	6.75	1027

Example applications (continued)

Aggregate analysis

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 the 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 6a). Both dimer aggregate and monomer are successfully detected (Figure 6b and 6c). Charge states are labeled in blue.

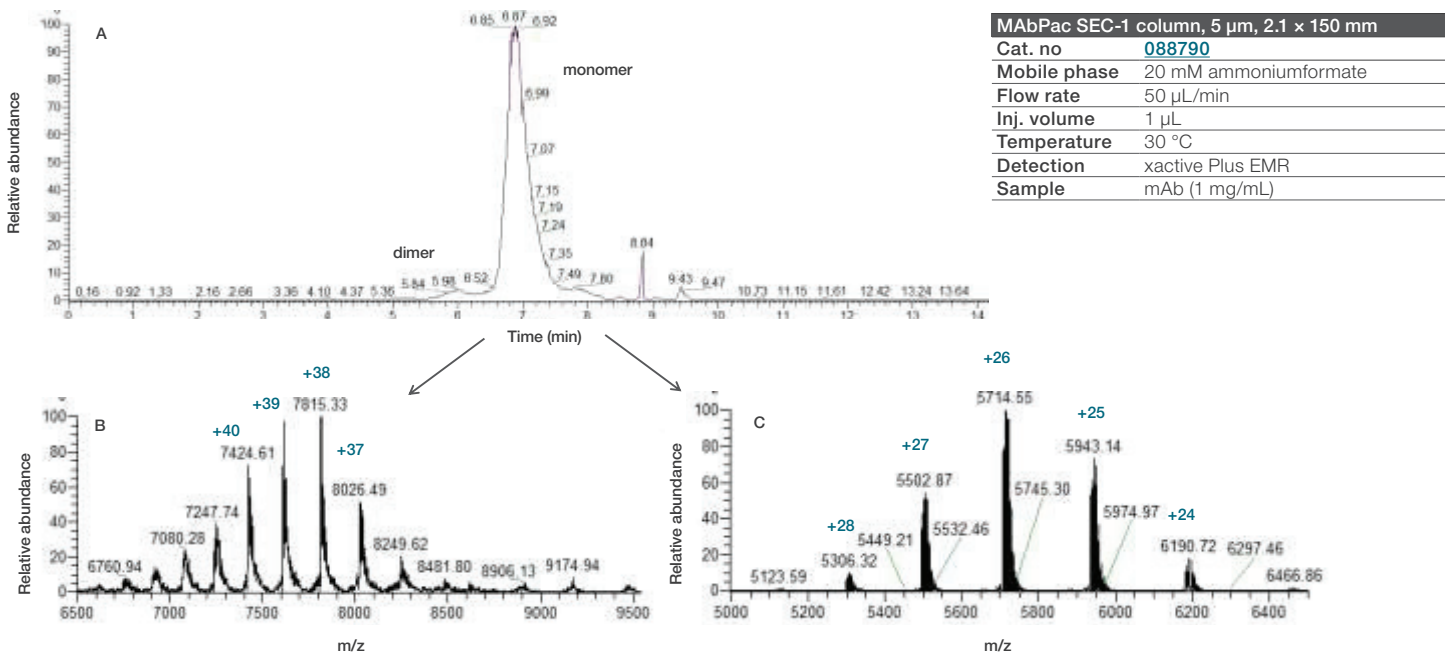


Figure 6. SEC-MS analysis of MAb dimer aggregates and monomer under non-denaturing condition

Frequently asked questions

How to achieve the best resolution on MAbPac SEC-1 column?

It is important to use the appropriate sample loop, tubing, and flow cell to achieve the best resolution. Smaller sample injection volume and reduced dead volume will improve the peak resolution. Refer to Table 3 in this manual for guidance.

What is the recommended buffer for the mAb and aggregate separation?

SEC silica is stable in the pH range of 2.5 to 7.5. The PBS buffer (50 mM sodium phosphate and 300 mM sodium chloride at pH 6.8) is most commonly used buffer for mAb and aggregate separation. We also recommend using 20 mM MES and 300 mM sodium chloride at pH 6.1.

What is the recommended volatile buffer for non-denaturing SEC-MS?

20 - 100 mM ammonium formate or ammonium acetate.

What is the recommended volatile buffer for denaturing SEC-MS?

20% acetonitrile and 0.1% formic acid and 0.05% trifluoroacetic acid.

What is the recommended storage condition for the MAbPac SEC-1 column?

For long term storage, we recommend storing the column in 20% acetonitrile.

Ordering information

Ordering information

Description	Quantity	Cat. no
MABPac SEC-1 analytical column SST 5 µm, 300 Å, 7.8 × 300 mm	Each	088460
MABPac SEC-1 analytical column PEEK 5 µm, 300 Å, 4.0 × 300 mm	Each	074696
MABPac SEC-1 analytical column PEEK 5 µm, 300 Å, 4.0 × 150 mm	Each	075592
MABPac SEC-1 guard column PEEK 5 µm, 300 Å, 4.0 × 50 mm	Each	074697
MABPac SEC-1 analytical column SST 5 µm, 300 Å, 2.1 × 300 mm	Each	088789
MABPac SEC-1 analytical column SST 5 µm, 300 Å, 2.1 × 150 mm	Each	088790

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