
# MAbPac SEC-1 Columns

065402 Revision 06 • October 2015

Thermo SCIENTIFIC

*For Research Use Only. Not for use in diagnostic procedures.*

# Product Manual

# for

# MAbPac SEC-1 Analytical Column

7.8 × 300 mm, (P/N 088460)
4 × 300 mm, (P/N 074696)
4 × 150 mm, (P/N 075592)
4 × 50 mm, (P/N 074697)
2.1 × 300 mm, (P/N 088789)
2.1 × 150 mm, (P/N 088790)

Revision History:

Revision 03, May 24, 2012, Reformatted for Thermo Scientific. Updated shipping solvent.

Revision 04, April, 2014, Formatting changes and grammatical corrections; clarification of operating conditions; harmonize advice in sections 1, 2 & 3 for column storage; revised cleaning procedures; up-to-date sample reports; reduced maximum pressure limits. Removed Sample QARs in the Appendix

Revision 05, June, 2014, Added three new column sizes and example applications.

Revision 06, October, 2015, Converted special part numbers to standard part numbers.

## Safety and Special Notices

Make sure you follow the precautionary statements presented in this guide. The safety and other special notices appear in boxes.

Safety and special notices include the following:

| | |
|---|---|
|  **SAFETY** | ***Indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.*** |
|  **WARNING** | ***Indicates a potentially hazardous situation which, if not avoided, could result in damage to equipment.*** |
|  **CAUTION** | ***Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. Also used to identify a situation or practice that may seriously damage the instrument, but will not cause injury.*** |
|  **NOTE** | ***Indicates information of general interest.*** |
| **IMPORTANT** | ***Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.*** |
| **Tip** | ***Highlights helpful information that can make a task easier.*** |

# Contents


**1. Introduction ..... 7**

1.1 Introduction to the MAbPac SEC-1 Column ..... 7

1.2 MAbPac SEC-1 Operating Limits and Specifications ..... 7

1.2.1 Operating Conditions ..... 7

1.2.2 Physical Characteristics ..... 8

1.2.3 Calibration Curve ..... 9

1.3 Formats of the MAbPac SEC-1 Columns ..... 10

**2. Getting Started; Step-By-Step Procedure ..... 11**

Step 1 – Visually Inspect the Column. ..... 11

Step 2 – Mobile Phase Selection. ..... 11

Step 3 – Set Up the LC System. ..... 11

Step 4 – Operational Guidelines ..... 12

Step 5 – Reproduce the Chromatogram in the Lot Validation Report ..... 12

Step 6 – Real sample Analysis ..... 13

**3. Column Care ..... 14**

3.1 Column Storage ..... 14

3.2 Operating pH Range: pH 2.5 to 7.5 ..... 14

3.3 Operating Temperature Limit: up to 30 ºC ..... 14

3.4 Pressure Limit: 1,000 psi for 300 mm Long Column; 600 psi for 150 mm Long Column ..... 14

3.5 Flow Rate ..... 14

3.6 Injection Volume ..... 14

3.7 Column Washing Procedure ..... 15

**4. Example Applications ..... 16**

4.1 Separation of mAbs and their Aggregates ..... 16

4.2 Separation of mAb Fragments ..... 19

4.3 Separation of mAbs Using High and Low Ionic Strength Eluents ..... 20

4.4 Separation of mAbs Using Volatile Buffers ..... 21

4.5 Ruggedness of MAbPac SEC-1 ..... 22

4.6 Aggregate Analysis ..... 23

**5. Frequently Asked Questions ..... 24**
5.1 How to achieve the best resolution on the MAbPac SEC-1 column? ..... 24
5.2 What is the recommended buffer for the mAb and aggregate separation? ..... 24
5.3 What is the recommended volatile buffer for non-denaturing SEC-MS? ..... 24
5.4 What is the recommended volatile buffer for denaturing SEC-MS? ..... 24
5.5 What is the recommended storage condition for the MAbPac SEC-1 column? ..... 24

# 1. Introduction

## 1.1 Introduction to the MAbPac SEC-1 Column

MAbPac SEC-1 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. Size Exclusion Chromatography (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 analtyes.

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 I.D. columns are packed into stainless steel housing while the 4.0 mm I.D. format columns are packed into PEEK housing.

## 1.2 MAbPac SEC-1 Operating Limits and Specifications

### 1.2.1 Operating Conditions

| Parameter | Recommendation |
|---|---|
| Flow Rate Range (recommended) | 760 – 1,000 µL/min for the 7.8 mm I.D. columns<br>200 – 300 µL/min for the 4.0 mm I.D. columns<br>50 – 75 µL/min for the 2.1 mm I.D. columns |
| Shipping Solution / Long Term Storage Solution | 20% acetonitrile in deionized |
| Typical buffers | Phosphate buffer with NaCl, e.g. 50 mM phosphate buffer (pH 6.8) + 0.3 M NaCl<br>Good's buffer with NaCl, e.g. 20 mM MES buffer (pH 6.1) + 0.3 M NaCl<br>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<br>600 psi for 150-mm long columns |
| pH Range | 2.5 – 7.5 |

**NOTE**

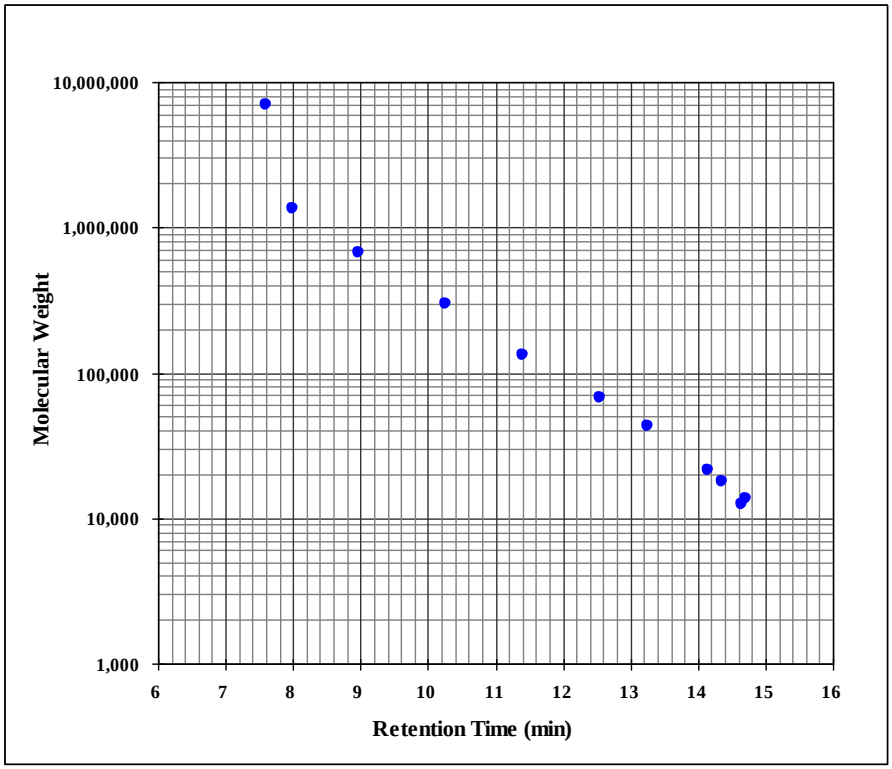
***Assistance is available for any problem during the shipment or operation of Thermo Scientific columns at techsupport.ccs@thermofisher.com***

### 1.2.2 Physical Characteristics

| | |
|---|---|
| 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<br>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 |

### 1.2.3 Calibration Curve

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




| | |
|---|---|
| Column: | MAbPac SEC-1 |
| Column size: | 4.0 mm I.D. × 300 mm |
| Sample: | 1. Thyroglobulin<br>2. γ-Globulin<br>3. BSA<br>4. Ovalbumin<br>5. Trypsin inhibitor<br>6. Myoglobin<br>7. RibonucleaseA<br>8. CytchromeC |
| Sample concentration: | 0.03% each |
| Injection volume: | 5 µl |
| Eluent: | 50 mM Sodium phosphate buffer (ph 6.8) + 0.3M NaCl |
| Flow rate: | 0.20 ml/min |
| Detection: | UV at 220 nm |
| Temperature: | 25 °C |

| | Protein | MAbPac SEC-1 Retention Time (min) | MW (kDa) |
|---|---|---|---|
| 1 | γ-Globulin aggregate | 7.6 | 7,000* |
| 2 | Thyroglobulin dimer | 8.0 | 1,338 |
| 3 | Thyroglobulin | 9.0 | 669 |
| 4 | γ-Globulin dimer | 10.3 | 300 |
| 5 | BSA dimer | 11.4 | 134 |
| 6 | BSA | 12.6 | 67 |
| 7 | Ovalbumin | 13.3 | 43 |
| 8 | Trypsin Inhibitor | 14.1 | 22 |
| 9 | Myoglobin | 14.4 | 18 |
| 10 | Ribonuclease A | 14.7 | 14 |
| 11 | Cytochrome C | 14.6 | 12 |

* Tentative, not actual

## 1.3 Formats of the MAbPac SEC-1 Columns

Currently, MAbPac SEC-1 size exclusion columns are available in 7.8 mm, 4.0 mm and 2.1 mm diameter formats.

| Product Description | Part Number |
|---|---|
| MAbPac SEC-1, 5µm, 300Å, Analytical column SST 7.8 × 300 mm | 088460 |
| MAbPac SEC-1, 5µm, 300Å, Analytical column PEEK 4.0 × 300 mm | 074696 |
| MAbPac SEC-1, 5µm, 300Å, Analytical column PEEK 4.0 × 150 mm | 075592 |
| MAbPac SEC-1, 5µm, 300Å, Guard column PEEK 4.0 × 50 mm | 074697 |
| MAbPac SEC-1, 5µm, 300Å, Analytical column SST 2.1 × 300 mm | 088789 |
| MAbPac SEC-1, 5µm, 300Å, Analytical column SST 2.1 × 150 mm | 088790 |

# 2. 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.

## Step 1 – 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.

## Step 2 – 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.

## Step 3 – 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 small internal diameter (ID) tubing (especially between the injector and detector) and a proper detector flow cell is required for best results. See Table 1 for recommend chromatographic set up. The system should be thoroughly primed before use. It is recommended the column is run at room temperature (20 to 30 °C) to achieve better column lifetime.

**Table 1 – Recommended Chromatographic Setup for MAbPac SEC-1**

| | | | |
|---|---|---|---|
| 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 | 100 | 150 – 250 |
| Sample Injection Size (µL) | 1 – 2 | 5 – 10 | 20 – 50 |

## Step 4 – Operational Guidelines

- Avoid any sudden pressure surge.
- Operate within column specifications (described in 1.2.1 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.
- Buffers are recommended during use of this column: typically a combined concentration of 20mM (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.

## Step 5 – 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.***

## Step 6 – Real sample Analysis

Once the column performance is satisfactorily confirmed in Step 5, 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 Section 3.7).***

# 3. Column Care

## 3.1 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.

## 3.2 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.

## 3.3 Operating Temperature Limit: up to 30 ºC

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 – 25 °C.

## 3.4 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.

## 3.5 Flow Rate

Please refer to Table 1 for recommended flow rate. Please note that the analyte efficiency may be affected at a higher flow rate.

## 3.6 Injection Volume

Please refer to Table 1 for recommended injection volume. Large injection volume may affect resolution.

## 3.7 Column Washing Procedure

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

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.

# 4. Example Applications

## 4.1 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 is specially designed for analysis of mAbs and their aggregates (Figure 1a, 1b and 1c). 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.

**Figure 1a –** Analysis of Monoclonal Antibody (mAb) and Aggregates (7.8 × 300 mm)

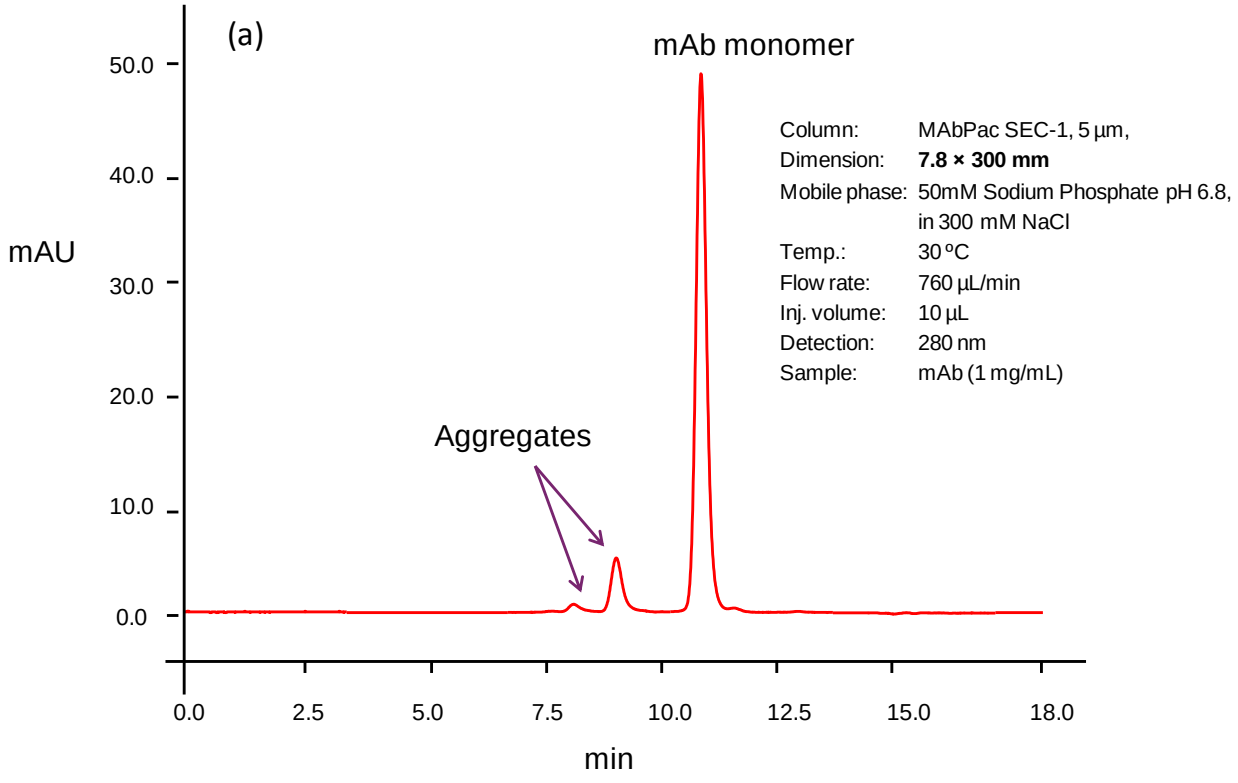


**Figure 1b –** Analysis of Monoclonal Antibody (mAb) and Aggregates (4.0 × 300 mm)

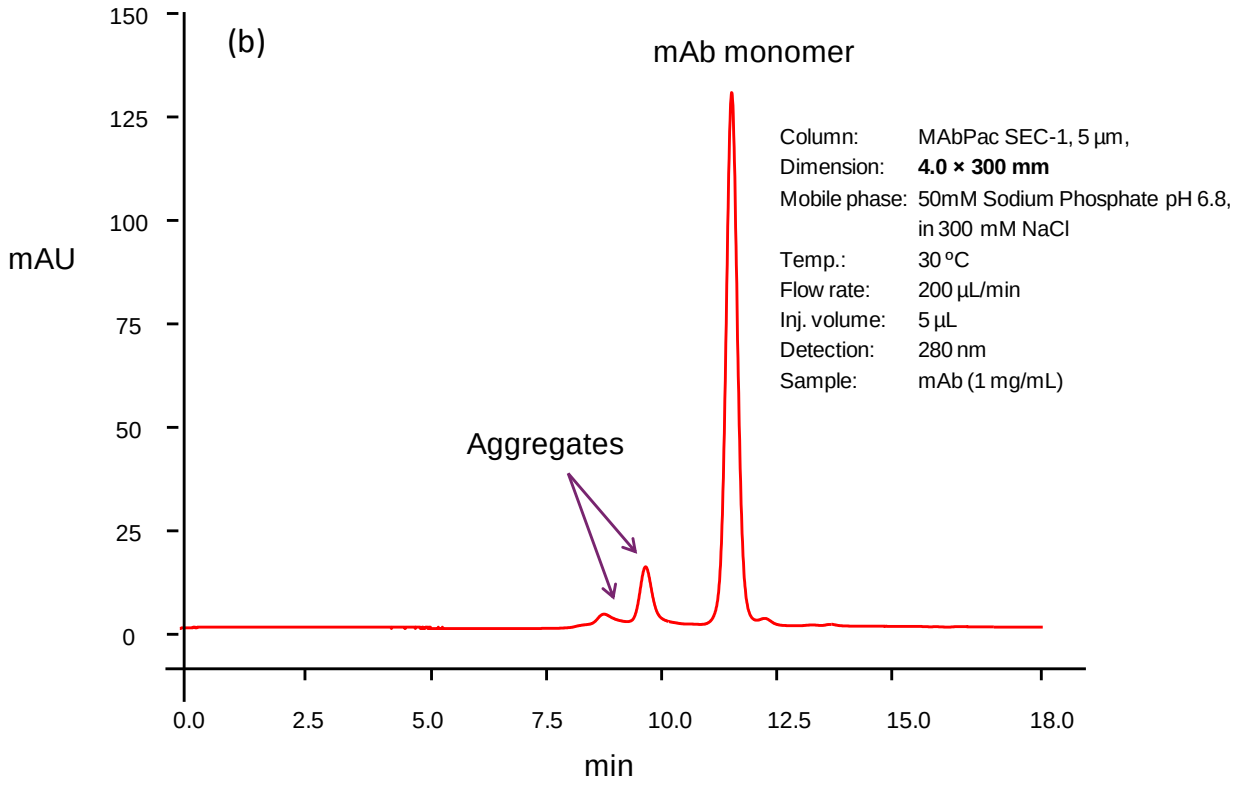
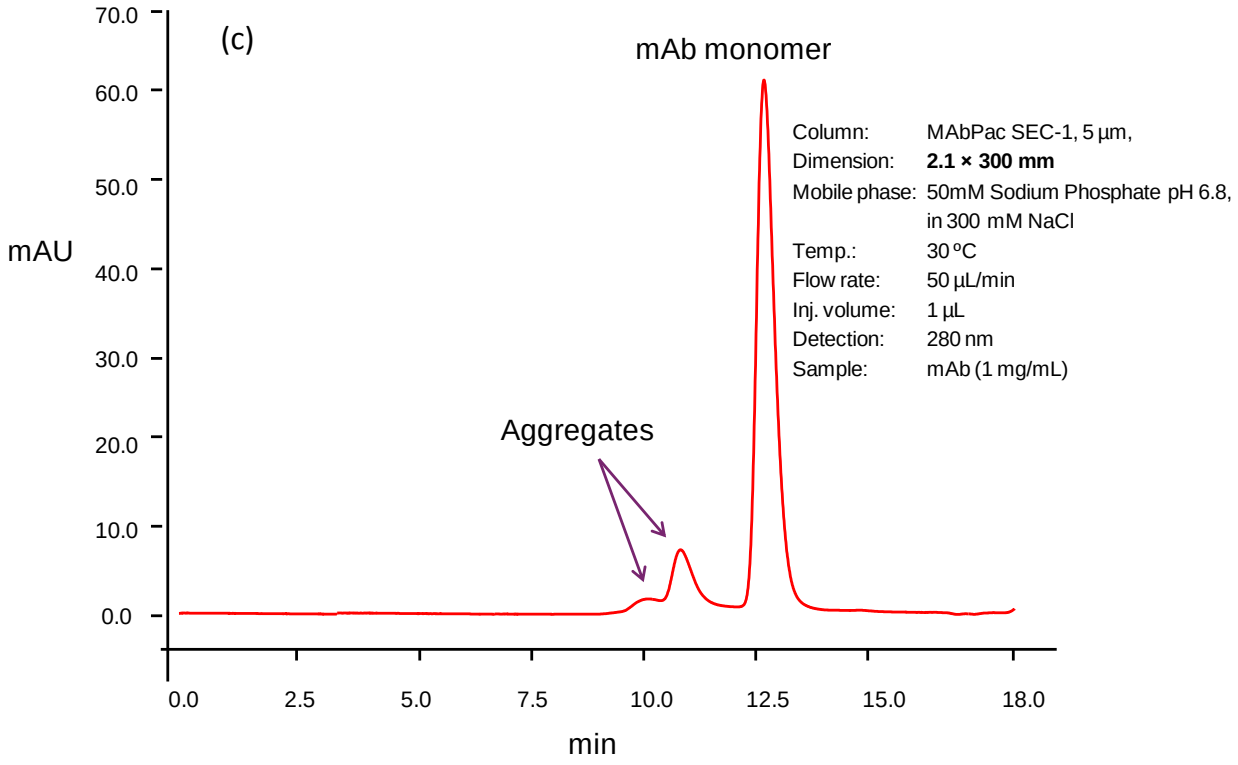


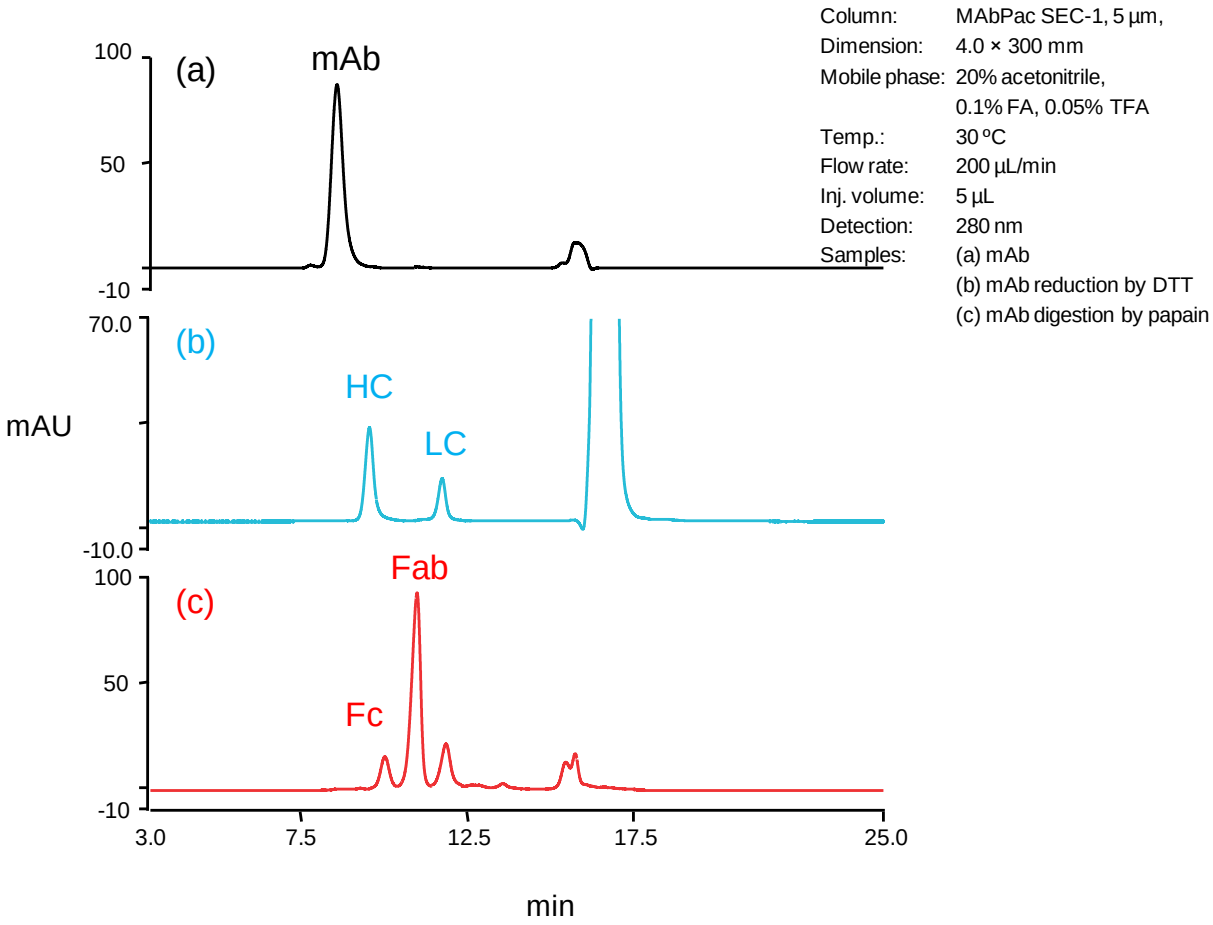
**Figure 1c –** Analysis of Monoclonal Antibody (mAb) and Aggregates (2.1 × 300 mm)



## 4.2 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.

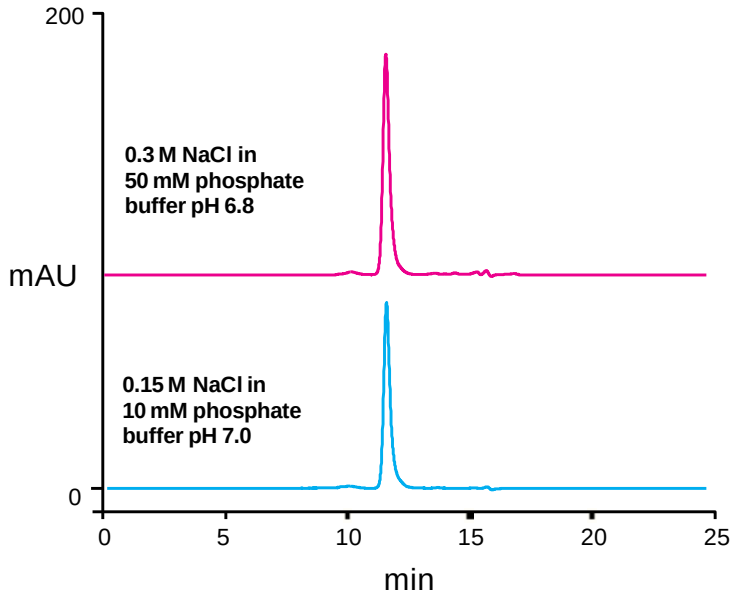
**Figure 2 –** mAb and mAb Fragments Analysis Using Denaturing Eluent



## 4.3 Separation of mAbs Using High and Low Ionic Strength Eluents

The MAbPac SEC-1 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.

**Figure 3 –** mAb Analysis using MAbPac SEC-1 in High-Salt and Low-Salt Eluents

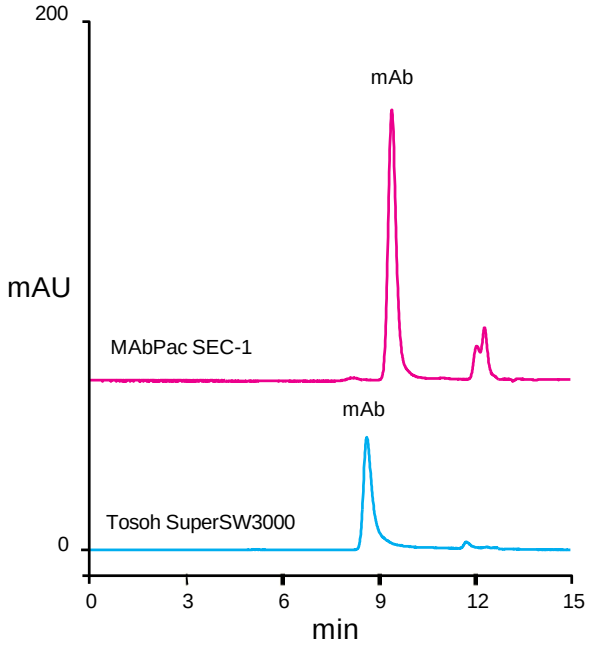



| | |
|---|---|
| Column: | MAbPac SEC-1, 5 µm, |
| Dimension: | 4.0 × 300 mm |
| Mobile phase: | 0.3 M NaCl in 50 mM phosphate buffer pH 6.8 (upper C-gram); 0.15 M NaCl in10 mM phosphate buffer pH 7.0 (lower C-gram); |
| Temp.: | 30 °C |
| Flow rate: | 200 µL/min |
| Inj. volume: | 5 µL |
| Detection: | 280 nm |
| Sample: | mAb (1 mg/mL) |

## 4.4 Separation of mAbs Using Volatile Buffers

The proprietary bonding chemistry of the MAbPac SEC-1 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

**Figure 4 –** mAb Analysis in Volatile Buffer MAbPac SEC-1 (Thermo) vs. SuperSW3000 (Tosoh)




| | |
|---|---|
| Column: | MAbPac SEC-1, 5 µm, 4.0 × 300 mm<br>Tosoh SuperSW3000, 4.6 × 300 mm |
| Mobile phase: | 0.1 M $NH_4OAc$, pH 5 |
| Temp.: | 25 °C |
| Flow rate: | 250 µL/min for MAbPac SEC-1<br>330 µL/min for Tosoh SuperSW3000 |
| Inj. volume: | 2.0 µL on MAbPac SEC-1<br>2.5 µL on Tosoh SuperSW3000 |
| Detection: | 280 nm |
| Sample: | mAb (1 mg/mL in buffer) |

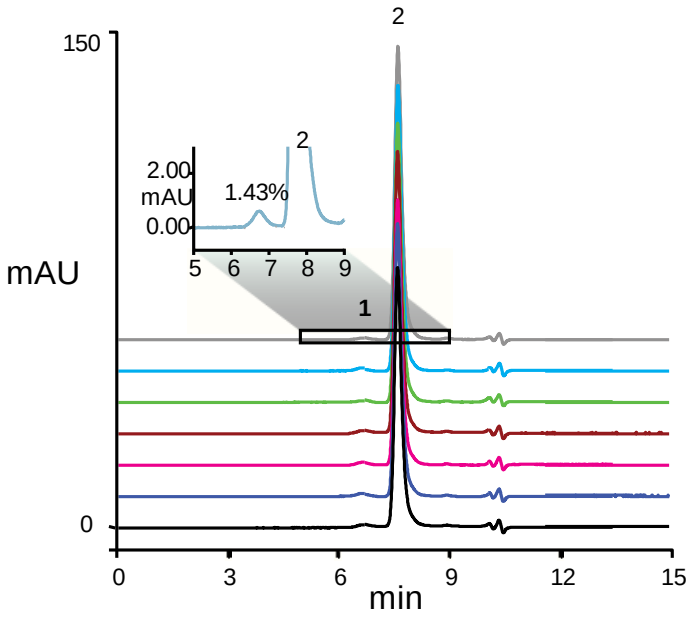
Note: Flow rate and injection volume are adjusted for the same linear velocity and relative loading to make fair comparison.

| | MAbPac SEC-1 (Thermo) | SuperSW3000 (Tosoh) |
|---|---|---|
| PW (50% height) | 0.256 min | 0.296 min |
| Efficiency (plates) | 6780 | 5005 |
| Asymmetry | 1.31 | 2.14 |
| Peak Height (mAU) | 105.3 | 43.7 |

## 4.5 Ruggedness of MAbPac SEC-1

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



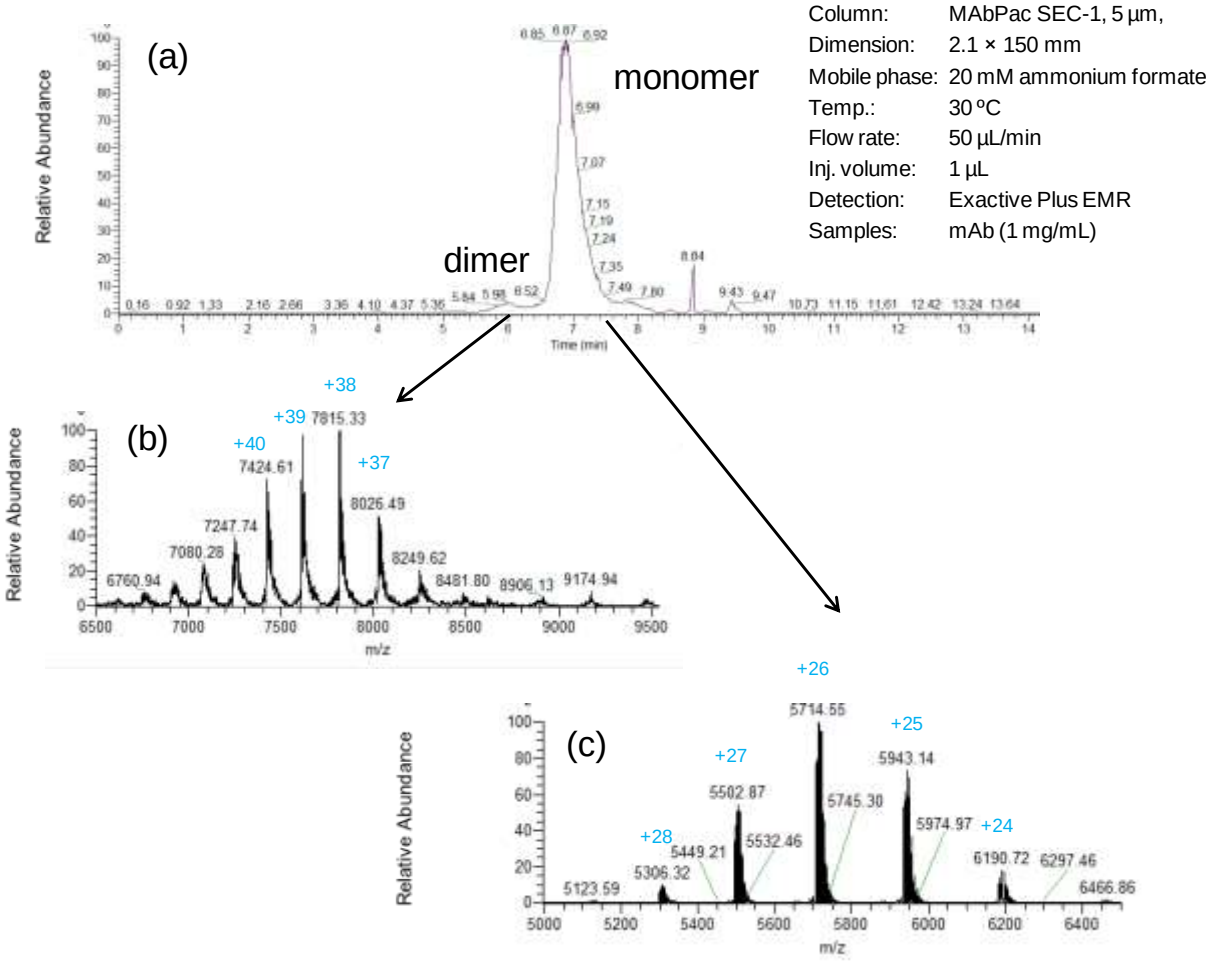

Column: MAbPac SEC-1, 5 µm,
Dimension: 4.0 × 300 mm
Mobile phase: 50mM Sodium Phosphate pH 6.8, in 300 mM NaCl
Temp.: 30 °C
Flow rate: 300 µL/min
Inj. volume: 5 µL
Detection: 280 nm
Sample: mAb (1 mg/mL)
Peaks: 1. mAb dimer
2. mAb monomer

| 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 |

## 4.6 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.



# 5. Frequently Asked Questions

## 5.1 How to achieve the best resolution on the 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 1 in this manual for guidance.

## 5.2 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.

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

20 - 100 mM ammonium formate or ammonium acetate

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

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

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

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