Thermo Scientific

MAbPac™ SEC-1 Columns

Product Manual

P/N: 065402-03 May 2012

Thermo SCIENTIFIC

Product Manual

for

MAbPac™ SEC-1 Analytical Column

(4 x 300 mm, P/N 074696)
(4 x 150 mm, P/N 075592)
(4 x 50 mm, P/N 074697)

Revision History:

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

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

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

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 size. On the MAbPac SEC-1 column, very large analytes (> 1000 kDa) are excluded by the pores thus eluting in the void, whereas smaller molecules (<1000 Da) pass through the pores and elute according to their size.

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 column is packed into non-metallic, bio-compatible, PEEK housing to eliminate compromised chromatography due to metal related inhibitory effects from the column hardware.

1.2 MAbPac SEC-1 Operating Limits and Specifications

1.2.1 Operating Conditions

Parameter	Recommendation
Flow Rate Range (recommended)	0.2 - 0.3 mL/min
Shipping Solution / Long Term Storage Solution	20% acetonitrile in deionized water / 300 mM NaCl in 50mM $NaPO_4$ buffer pH 6.8 containing 0.1% NaN_3 or 20% ethanol
Typical buffers	50 mM phosphate buffer (pH 6.8) + 0.3 M NaCl, PBS and other commonly used buffers
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	Ambient - 30 °C
Pressure Limit	1,500 psi.
pH Range	2.5 – 7.5

NOTE

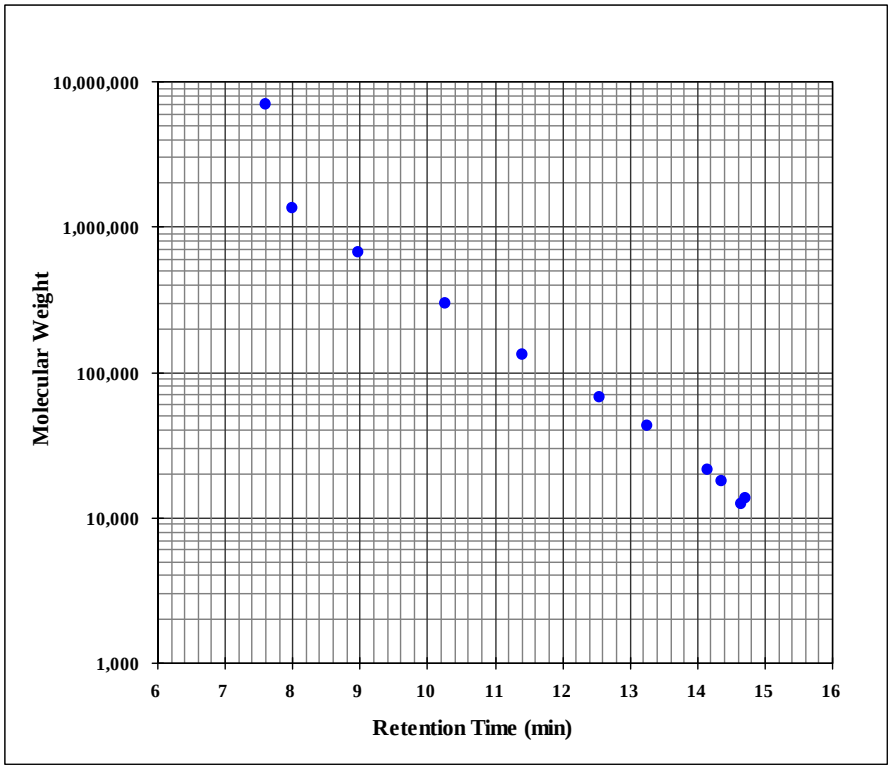
Assistance is available for any problem during the shipment or operation of Thermo Scientific columns at techsupport.ccs@thermofisher.com

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
Separation range for globular proteins	10,000 - 1,000,000
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. x 300 mm
Sample:	1. Thyroglobulin 2. γ-Globulin 3. BSA 4. Ovalbumin 5. Trypsin inhibitor 6. Myoglobin 7. RibonucleaseA 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	Ovalubumin	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 4 x 300 mm, 4 x 150 mm and 4 x 50 mm formats.

Product Description	Part Number
MAbPac SEC-1, 5µm, 300Å, Analytical column PEEK 4.0 x 300 mm	074696
MAbPac SEC-1, 5µm, 300Å, Analytical column PEEK, 4.0 x 150mm	075592
MAbPac SEC-1, 5µm, 300Å, Guard column PEEK 4.0 x 50 mm	074697

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 6.0 - 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 acetate buffer can also be used when 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 prefiltered 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 highly recommended that the system be optimized for low dead volume; usage of small internal diameter (ID) tubing (0.005” I.D. tubing, especially between the injector and detector) and a semi-micro detector flow cell is required for best results. 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.

Step 4 – Operational Guidelines

- 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”).
- A new column is shipped in 20% acetonitrile in deionized water..
- 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.
- Always run blanks to make sure the protein of interest is not sticking onto the column and has not been carried over to the next run. If you find that a particular analyte showed carry-over on the next run, repeat a sufficient number of blank runs before you continue another sample injection. You may need to investigate suitability of the eluents used.
- Avoid use of the column below pH 2.5 or above pH 7.5.
- Maximum operating pressure is 1,500 psi.
- The recommended maximum operation temperature is 30 °C.
- Columns should not be used or stored under elevated basic condition (pH >7.0) or at elevated temperatures (> 30 °C).
- 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. Use low percentage (20%) organic solvent to wash off residual salts 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 access 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 0.1% NaN_3 or 20% ethanol to prevent microbial growth.

3.2 Operating pH range: pH 2.5 to 7.5

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

3.3 Operating temperature limit: 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: 1,500 psi for 4 x 300 mm format; 800 psi for 4 x 150 mm format

It is extremely important not to impose a sudden column pressure surge. Although the column pressure is rated up to 1500 psi for 4 x 300 mm format, the typical operating pressure at 0.30 mL/min should not exceed 1200 psi.

3.5 Flow rate

Flow rates of 0.2 mL/min to 0.3 mL/min are used for the MAbPac SEC-1 columns, while a higher flow rate (up to 0.4 mL/min) can be used for faster analysis as long as the pressure limit is not exceeded. Please note that the analyte efficiency may be affected at a higher flow rate.

3.6 Injection Volume

For a 4.0 x 300 mm column, the typical injection volume is about 1 to 10 µL. When this volume is increased it may affect the chromatography profile.

3.7 Column washing procedure

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 0.2 mL/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 um filter prior to use. In the event that column washing/cleaning is needed, the following procedure can be used as a guideline:

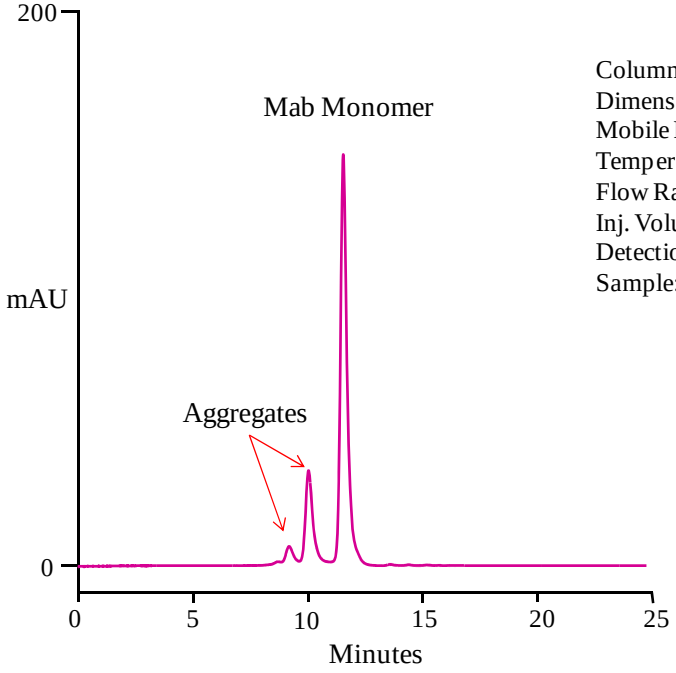
1. Wash the column with 20% methanol at 0.2 mL/min for 30 minutes.
2. Wash the column with 80% methanol at 0.2 mL/min for 30 minutes.
3. Wash the column with 100% water at 0.2 mL/min for 30 minutes.
4. To remove ionic contaminants flush the column with concentrated salt solution at low pH (e,g, 0.5 M Na_2SO_4 at pH 2.7) at 0.2 mL/min for 30 minutes.
5. Flush the column with buffered solution of SDS, urea, or guanidine, at 0.2 mL/min for 30 minutes.
6. Wash the column with 100% water at 0.2 mL/min for 30 minutes.
7. Wash the column with the mobile phase at 0.2 mL/min for at least 30 minutes.

4. Example Applications

4.1 Separation of Monoclonal Antibodies 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 1). 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.

Figure 1 – Analysis of Monoclonal Antibody (Mab) and Aggregates

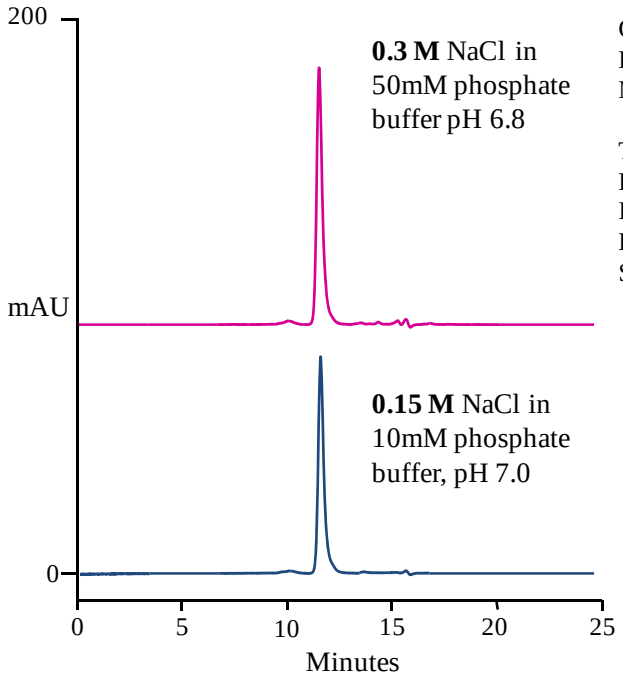


Column:	MAbPac SEC-1, 5 µm
Dimension:	4.0x300 mm (PEEK)
Mobile Phase:	0.3 M NaCl in 50mM phosphate buffer pH 6.8
Temperature:	30 C
Flow Rate:	0.20 mL/min
Inj. Volume:	2 µL
Detection:	UV, 280 nm
Sample:	MAb (10 mg/mL)

4.2 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 2, separation of MAb with MAbPac SEC-1 under both high salt condition (0.3 M NaCl in 50 mM phosphate buffer, upper panel) and low salt condition (0.15 M NaCl in 10 mM phosphate buffer, lower panel), displayed good peak shape and peak efficiencies.

Figure 2 – MAb Analysis using MAbPac SEC-1 in High-Salt and Low-Salt Eluents

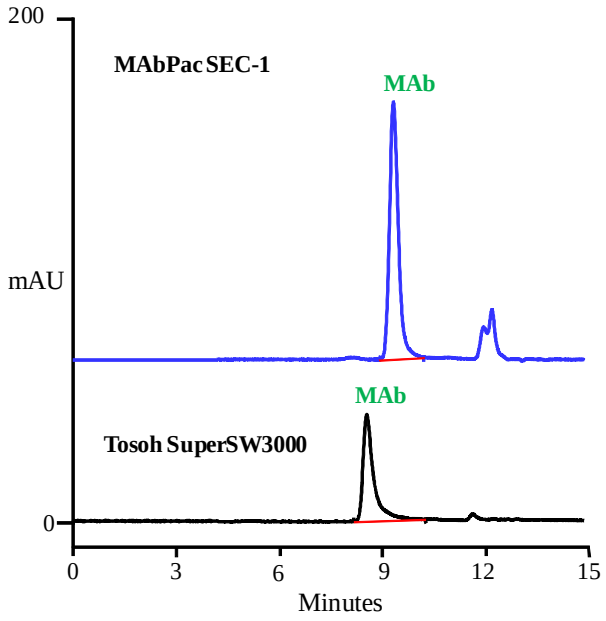


Column:	MAbPac SEC-1, 5 µm
Dimension:	4.0x300 mm (PEEK)
Mobile Phase:	0.3 M NaCl in 50mM phosphate buffer pH 6.8
	0.15 M NaCl in 10mM phosphate buffer, pH 7.0
Temperature:	30 C
Flow Rate:	0.20 mL/min
Inj. Volume:	5 µL
Detection:	UV, 280 nm
Sample:	MAb (1 mg/mL)

4.3 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 3 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 3 – MAb Analysis in Volatile Buffer MAbPac SEC-1 (Dionex) vs. SuperSW3000 (Tosoh)



Column: 4.0x300 mm, 5 µm
Mobile Phase: 0.1 M NH_4OAc, pH5 buffer
Temperature: 25 C
Flow Rate: 0.25 mL/min for MAbPac SEC-1
0.33 mL/min for Tosoh SuperSW3000
Inj. Volume: 2.0 µL on MAbPac SEC
2.5 µL on Tosoh SuperSW3000
Detection: UV, 280 nm
Sample: MAb (1 mg/mL in buffer)

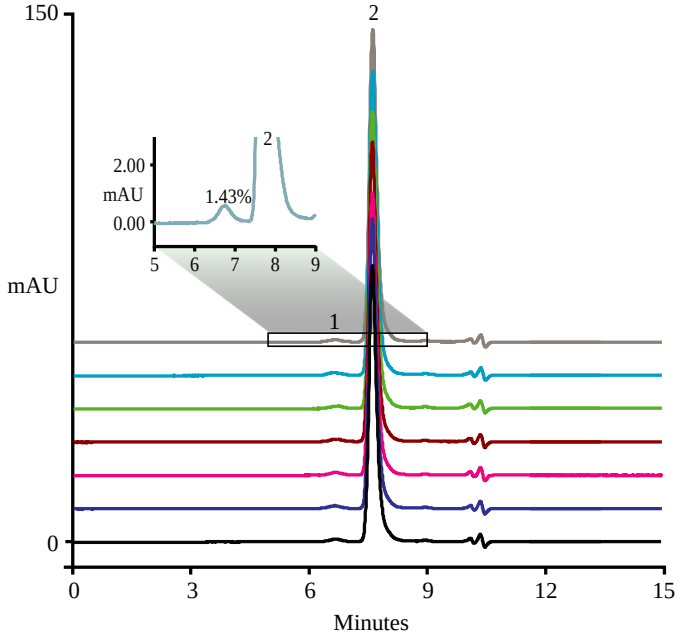
Note: Flow rate and injection volume are adjusted for the same linear velocity and relative loading to make fair comparison.

	MAbPac SEC (Dionex)	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

4.4 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 4 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 4 – Excellent Ruggedness for MAb Analysis



Column: MAbPac SEC-1, 5 µm
Dimention: 4.0 × 300 mm (PEEK)
Mobile Phase: 0.3 M NaCl in 50 mM phosphate buffer pH 6.8
Temperature: 30 °C
Flow Rate: 0.30 mL/min
Inj. Volume: 5 µL
Detection: UV, 280 nm
Sample: MAb (1 mg/mL)

Peaks: 1. Aggregates
2. MAb

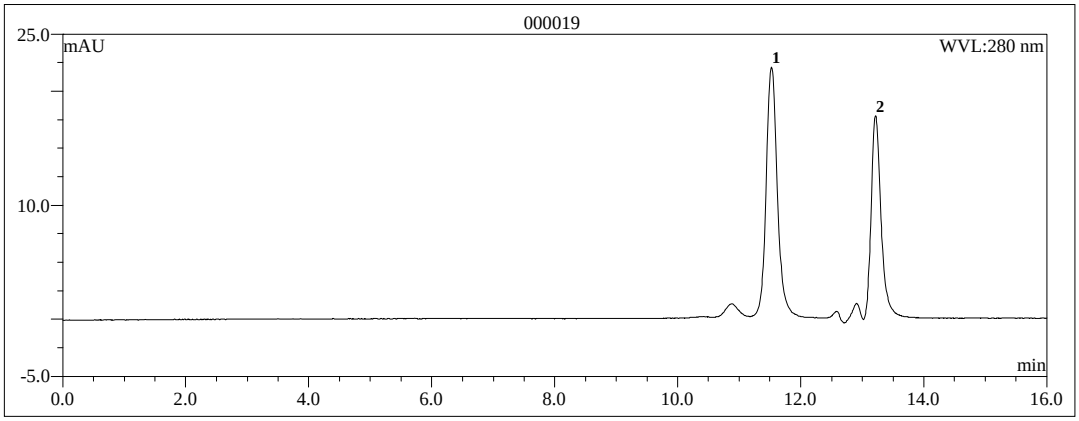
Percentage of Dimer Present in the MAb Sample					
Injection #	Monomer Retention Time	Asymmetry (10%)	Efficiency (Plates)	Dimer Retention Time	Pressure (psi)
10	7.71	1.39	7287	6.75	1017
100	7.71	1.36	7333	6.75	1020
160	7.71	1.37	7310	6.75	1020
250	7.71	1.35	7321	6.75	1027
319	7.71	1.33	7311	6.75	1023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

5. Appendix – Example Column QC Performance Test Report

MAbPac™ SEC-1
5µm 300Å (4 X 300 mm), Analytical
Product No. 074696

Date: **20-Jul-10 18:21**
Serial No. : **000019**
Lot No. : **05-16**

Mobile Phase: 50 mM Sodium Phosphate pH 6.8 + 300 mM NaCl
Flow Rate: 0.25 mL/min **Injection Volume:** 5.0 µL
Detection: 280 nm **Temperature:** 25 °C
Shipping Solution: 20% acetonitrile in DI wate r



No.	Peak Name	Ret.Time (min)	Asymmetry (EP)	Efficiency (EP)	Concentration
1	Myoglobin (Horse heart)	11.5	1.14	21821	0.25 mg/mL
2	Cytidine	13.2	1.29	35493	8 µg/mL

QA Results:

Analyte	Parameter	Specification	Results
Cytidine	Retention Tim e	12.1-14. 1	Passed
Cytidine	Asymmetry	0.90-1.5 6	Passed
Cytidine	Efficienc y	>=23,85 0	Passed
Myoglobin (Horse heart)	Efficienc y	>=14,40 0	Passed
	Pressur e	<=110 0	743

Production Re ference:
Datasource : QAR
Directory: MAbPac\SEC-1
Sequence: SEC-1 _4x300 _2-Mix _Ind_Test
Sample No: 1

6.80 SR11 Build 3161 (184582) (Demo-Installation)

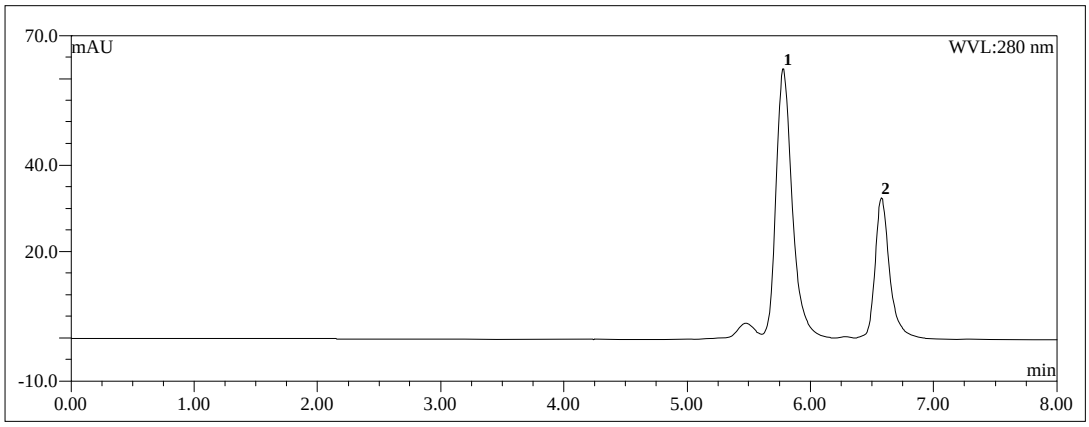
Chromeleon™ Thermo Fisher Scientific

074634-02 (QAR)

MAbPac™ SEC-1
5µm 300Å (4 X 150 mm), Analytical
Product No. 075592

Date: **05-Nov-10 07:11**
Serial No. :
Lot No. : **30-05-18**

Mobile Phase: 50 mM Sodium Phosphate pH 6.8 + 300 mM NaCl
Flow Rate: 0.25 mL/min **Injection Volume:** 5.0 µL
Detection: 280 nm **Temperature:** 25 °C
Shipping Solution: 20% acetonitrile in DI wate r



No.	Peak Name	Ret.Time (min)	Asymmetry (EP)	Efficiency (EP)	Concentration
1	Myoglobin (Horse heart)	5.8	1.3	10449	0.25 mg/mL
2	Cytidine	6.6	1.4	17200	8 µg/mL

QA Results:

Analyte	Parameter	Specification	Results
Cytidine	Retention Tim e	6.0-7.1	Passed
Cytidine	Asymmetry	0.9-1.7	Passed
Cytidine	Efficiency	>=10,800	Passed
Myoglobin (Horse heart)	Efficiency	>=6,300	Passed
	Pressure	<=550	303

Production Re ference:
Datasource : QAR
Directory: MAbPac\SEC-1
Sequence: SEC-1_4x150_2-Mix_Ind_Test
Sample No: 1

6.80 SR11 Build 3161 (184582) (Demo-Installation)

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