Waters™

GTxResolve Premier SEC 1000 Å 3 µm Columns

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Please pay special attention to information on:

- MALS column preparation
- Flow cells and their effect on recovery and peak shape
- LNP method considerations

I. INTRODUCTION

Thank you for choosing a Waters™ SEC Column. GTxResolve™ Premier SEC 1000 Å, 3 µm Columns help scientists obtain fast and reliable size variant analyses of gene therapeutics (GTx), as made possible with Waters MaxPeak™ High Performance Surfaces (HPS) and a rugged widepore particle and novel crosslinked HO-PEO bonding technology (Figures 1 and 2). The packing material of the GTxResolve Premier SEC 1000 Å, 3 µm Column is a high strength silica particle that is further modified with a polyethylene oxide bonding and bridged ethylene hybrid crosslinks. This new particle technology exhibits industry leading inertness toward both ionic and hydrophobic secondary interactions. It is also carefully designed to impart increased pH resilience and reduced levels of chemical noise. SEC with these hardware and particle technologies can be advantageously used to characterize and quantify the soluble aggregates and product related degradants of a gene therapeutic. The lower secondary interactions and background noise of these columns contribute to improved compatibility with universally applied detectors, such as multiangle light scattering (MALS).

Advancements in SEC column hardware and particle technology work to minimize secondary interactions between analytes and the column to allow chromatographers the chance to develop robust multiattribute platform methods for aggregate, titer, and integrity measurements with options for 3x reduced sample consumption, 2x higher throughput, and 2x improved sensitivity with MALS. Optimum separation performance is achieved when 4.6 mm ID columns are paired with low dispersion UPLC systems. High resolution SEC separations are most easily achieved on higher dispersion HPLC systems through the use of a 7.8 mm ID column.

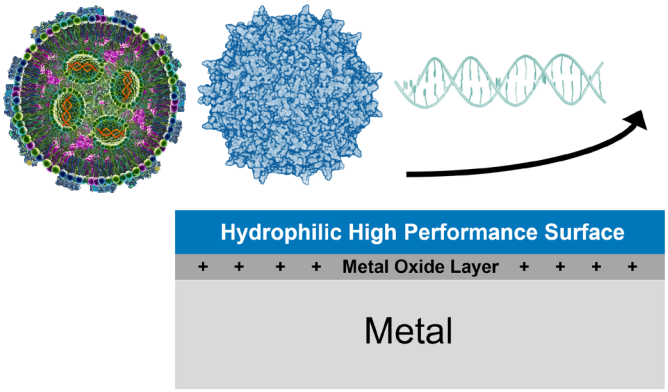

Figure 1. A MaxPeak High Performance Surface with hydrophilic properties to minimize secondary interactions between biomolecules and column hardware. PDB: 1LP3.

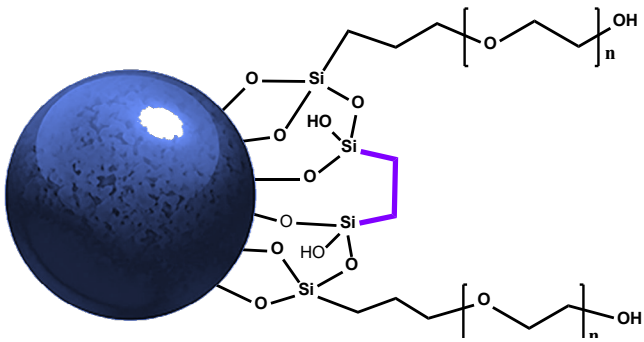

Figure 2. A schematic representation of the hybrid crosslinked HO-PEO bonding of the GTxResolve Premier SEC 1000Å 3 µm Column.

A Waters GTxResolve Premier SEC 1000 Å, 3 µm Guard Column is also available, which can provide effective trapping of insoluble particulates and excipients sometimes present in samples and eluents, thereby extending the analytical column's lifetime. One single, low dispersion 4.6 x 30 mm design is available for use with both 4.6 and 7.8 mm ID analytical columns.

GTxResolve Premier SEC 1000 Å, 3 µm Guards are manufactured in a cGMP, ISO 9001 certified plant using stringent manufacturing protocols and ultra-pure reagents. Each batch of GTxResolve Premier SEC 1000 Å packing material must pass a series of stringent QC tests that includes testing with the Waters dsDNA 50 to 1350 Ladder as well as with a protein mixture containing thyroglobulin and its dimer.

II. SEC ANALYSIS OF NUCLEIC ACIDS AND PROTEIN MIXTURE

A. CONSIDERATIONS

Historically, size-exclusion chromatography (SEC) has been widely used to assess non-covalent protein aggregation (high molecular weight species [HMWS]). It is a technique that has been relied upon for years to support the development of recombinant protein drugs. SEC is now being adopted and optimized for performing aggregate and sizing analyses on LNPs, mRNA and viral vectors. Example chromatograms obtained for the Waters dsDNA 50 to 1350 Ladder P/N: 186010778 and Waters SEC Protein Standard Mix P/N: 186006842 are shown in Figures 3A & 3B.

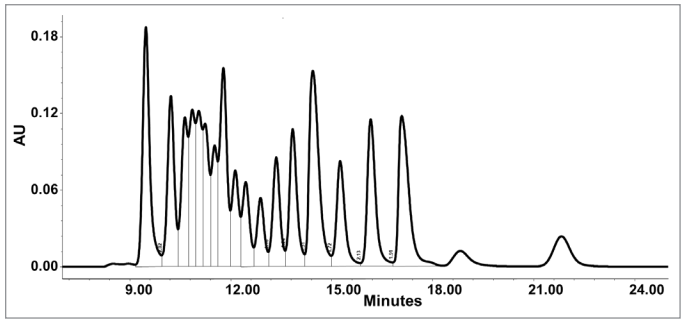

Figure 3A. Example Waters dsDNA 50 to 1350 Ladder (P/N: 186010778) chromatogram as obtained with an GTxResolve Premier SEC 1000 Å, 3 µm 4.6 x 150 mm column using a flow rate of 0.10 mL/min and a mobile phase comprised of 20 mM sodium phosphate, 276 mM NaCl, 5.4 mM KCl pH 7.4, Temperature: 35°C; Detection: UV 260 nm.

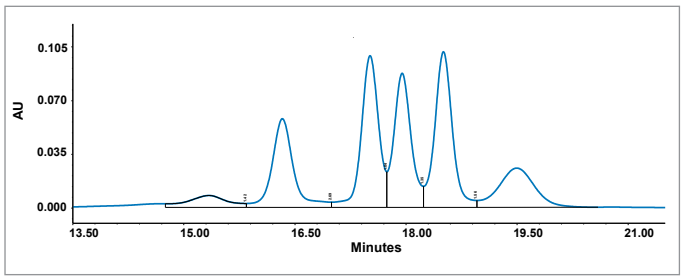

Figure 3B. Example Waters Protein Standard Mix (P/N: 186006842) chromatogram as obtained with a GTxResolve Premier SEC 1000 Å, 3 µm 4.6x150 mm column using a flow rate of 0.10 mL/min and a mobile phase comprised of 20 mM sodium phosphate, 276 mM NaCl, 5.4 mM KCl pH 7.4, Temperature: 35 °C; Detection: UV 280 nm.

Resolution between the closely eluting peaks is possible due to the high efficiency particles, quality of the packed bed, and the incorporation of low adsorption surfaces. Shear forces have been a concern of many when it comes to applying SEC for gene therapeutics. In practice, little to no shear effects have been observed with the use of GTxResolve Premier SEC 1000 Å, 3 µm SEC Columns.

Nevertheless, it is advised to apply reason when optimizing the flow rate of a new separation. Lower flow rate analyses are desirable for their high-resolution outcomes, and they are hypothetically less likely to induce shear effects. To achieve higher throughput separations, it is advised to take advantage of 150 mm length columns.

B. UNDERSTANDING EXTRA-COLUMN SYSTEM DISPERSION

System dispersion is an important consideration when performing SEC. Very low band spread systems provide the best efficiency, especially if smaller ID, shorter columns are used. The apparent performance of 4.6 mm ID columns will be significantly hindered if ever used with a higher dispersion system. When using higher band spread HPLC and UHPLC systems, it should be noted that very good results can still be obtained by using longer and larger I.D. columns, such as a 7.8 x 300 mm length column.

In SEC separations, analytes elute within a single SEC column volume. As a result, it is important to understand the bandspread of your LC system (without the SEC column attached). This will tell you information about the system injector, post injector tubing, detector flow cell, and the quality of your tubing connections. The following procedure can be used to determine LC system dispersion volume, often referred to as the 5-Sigma band spread: Replace the column with a Zero-Volume Union (P/N: 700002636) and connect it to your UV detector with a piece of 0.0025'' x 8.5'' tubing (P/N: 700009971).

1. Purge all LC solvent, wash, and purge lines with water, then 50/50 water/acetonitrile.
2. Set the detector to 273 nm and collect data at >40 points per second with a filter setting of none.
3. Flow rate: 0.5 mL/min and equilibrate the system for 10 min.
4. Set run time to 1 min.
5. Prepare 0.16 mg/mL caffeine in 50/50 water/acetonitrile and set injection volume to 0.5 µL.
6. Inject 3 mobile-phase blanks followed by 5 caffeine sample injections.
7. Calculate the LC system volume by (a) measuring the caffeine peak width at 4.4% peak height, (b) multiplying the peak width by the flow rate to determine the peak volume width in µL.

Note: in general, a 4.6 mm ID SEC column is best used on an LC system with a bandspread volume of <25 µL. A 7.8 mm mm ID SEC columns is tolerant of LC system bandspread volumes of up to 50 µL.

C. OPTIMIZATION OF MALS DETECTION

SEC analysis of gene therapeutics is often hyphenated to Multi-Angle Light Scattering (MALS). MALS detection is both sensitive and informative. For instance, the aggregates within a viral vectored gene therapy sample can often be detected more sensitively by MALS than by UV detection. Moreover, MALS detection can simultaneously provide radius of gyration (Rg) of the analyte and aggregates as well as accurate quantitation of the aggregate concentration. Comprehensive characterization of viral vector samples is thereby facilitated because it becomes possible to quickly report empty/full ratios of the monomer and provide detailed information about the nature of the sample's high molecular weight species. In addition, similar types of multiattribute quantitation approaches are now coming to be established for lipid nanoparticles and their nucleic acid payloads.

GTxResolve Premier SEC 1000 Å, 3 µm Columns provide industry leading MALS sensitivity. For best results, column flushing prior to light scattering is required. SEC columns require up to 15 hours of flushing before they are ready for use with a MALS detector. The specialized compositions and manufacturing processes of the GTxResolve Premier SEC 1000 Å, 3 µm Columns ensure they are ready for MALS hyphenation after flushing to waste with minimum of 40 column volumes of mobile phase (see below).

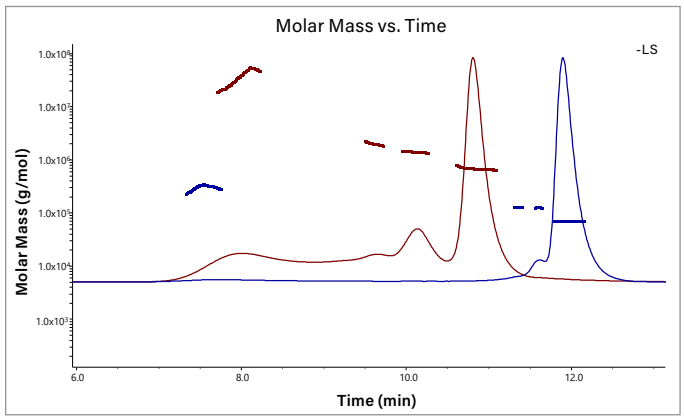

Figure 4. shows an example light scattering chromatogram generated on a GTxResolve Premier SEC 1000 Å 3 µm, 4.6 x 300 mm column for a sample comprised of bovine serum albumin (2 mg/mL, blue trace) and bovine thyroglobulin (4.5 mg/mL, brown trace) using 2x strength PBS buffer at 0.3 mL/min. These samples frequently contain significant quantities of dimer and trimer species. Bovine serum albumin can be used to confirm system suitability. Bovine thyroglobulin is shown here because it is an even better fit to the fractionation range of the GTxResolve Premier SEC 1000 Å 3 µm columns.

System Readiness for MALS Applications

Prior to sample analysis by MALS, it is important to assess the chromatographic instrument and MALS detector. The cleanliness of these instruments is measured by observing the noise level of the instrument without having an SEC column in the LC flow path. A MALS detector is highly sensitive to the presence of particulates, which can lead to noisy baselines, obscured sample signal and poor data quality. A noisy baseline and increased baseline voltage indicate contamination of the LC system or flow cell or both. Wandering and drifting baseline with excessive noise also indicates a dirty flow cell. High background noise means decreased sample sensitivity and incorrect molar mass determination. The baseline performance of a detector varies from instrument to instrument. The user is advised to refer to their detector's manual.

In general, the LC system should be flushed and cleaned at least quarterly according to the Waters SEC Optimization Guide' or sooner when increased noise levels are noticed.

Guidelines for system cleaning and noise assessment are also available from Wyatt (TN3501). It should be noted that an ACQUITY™ Premier or ARC™ Premier LC system's injector and active preheater before flushing with the recommended 10% phosphoric acid solution. Remedial measures to ensure low MALS noise include replacing the solvent inline filters and mixers every six months.

Mobile Phases for MALS:

Use high quality 18.2 MΩ purified water for buffer preparation. Thoroughly clean the mobile phase bottle and rinse it with purified water. Filter the prepared buffer through a disposable 0.1 µm filter. Watch for the expiration of buffers and avoid refilling partially filled reservoirs.

Column Preparedness for MALS (SPECIAL NOTE):

For best results, column flushing prior to light scattering is required. To avoid contaminating the light scattering detector, it is highly recommended to flush column offline before connecting to the light scattering detector due to sensitivity of foreign particulates or solvent incompatibility.

It is recommended to flush a new or used column for a minimum 40 column volumes before connecting it to a LS detector (See Table 1). Flush effluent can be collected directly into a waste container. Be gentle with introducing flow and step the flow rate at no more than 0.1 mL/min for 7.8 mm and 0.05 mL/min for 4.6 mm diameter columns. Columns are shipped and to be stored in a storage solution containing some organic content to minimize microbial growth. Flush no more than 1 column volume with 18.2 MΩ purified water before flushing with a buffer.

A typical regime of flush volumes is listed in ***Table 1***, For best performance, it is recommended to ready both previously used and brand-new columns with these same considerations.

Ramp Rate (mL/min)	Flow Rate (mL/min)*	Column Configurations	Column Volume**
0.05	0.40	4.6 x 30 mm	40
0.05	0.40	4.6 x 150 mm	40
0.05	0.40	4.6 x 300 mm	40
0.1	1.00	7.8 x 150 mm	40
0.1	1.00	7.8 x 300 mm	40

Table 1. Recommended flush conditions to equilibrate column for SEC-MALS experiments. Consideration of flushing new GTxResolve Premier SEC 1000 Å 3 µm Column. Here, a 1x PBS (10 mM sodium phosphate, 2.7 mM KCl, 137 mM NaCl) buffered saline mobile phase used was filtered with a 0.1 µm disposable filter.
**Remember to use operating flow rate and flush to recommended column volume.*
***Recommended minimum flushing column volumes to waste. If noise is still high, flush in 40CV increments.*

It is important to note that the column should be flushed to the minimum required column volume at the operating flow rate of your assay. For instance, if assay flow rate is 0.30 mL/min, flush column to waste at 0.30 mL/min for minimum of seven (7) hours before connecting to light scattering detector.

This flushing ensures column storage solution and possible sample contaminants have been thoroughly removed from the column before SEC-MALS (see below).

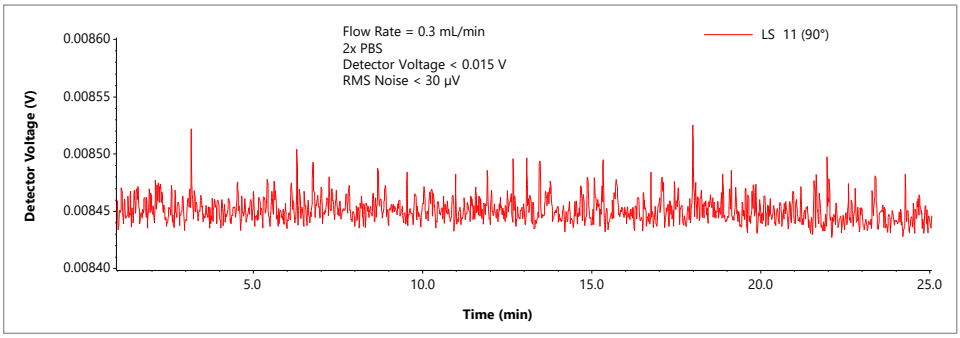

Figure 5: Example of a 4.6 x 300 mm ID GTxResolve Premier SEC 1000 Å 3 µm Column showing RMS noise < 30 µV using a 0.30 mL/min flow rate with 2x PBS (20 mM sodium phosphate, 5.4 mM KCl, 274 mM NaCl) buffered saline mobile phase after 40 CV flushing. WYATT DAWN MALS detector example.

Column Connections and Readiness:

Once the column has been flushed, and the instrument is in the ready status, the flushed column can be connected to the LS detector provided the buffer used to flush the column is identical. If the preparation is different from that used to flush the column, disconnect the tubing to detectors and cap the union to prevent air from entering the in-line detectors. Connect the tubing from chromatographic system to the column inlet and connect tubing from the column outlet to a waste container. The column can then be flushed to waste. Essentially, this process is to transition the flush solution to the running buffer in which the detector has been ready to.

Confirming SEC-MALS system suitability:

The WYATT DAWN MALS detector has a display screen to indicate its readiness status. It illuminates in green or orange or red depending on its measured noise levels. A green, or 'good-to-go', status was achieved upon the installation and 40 CV flushing of a GTxResolve Premier SEC, 1000Å, 3 µm Column (as shown in Figure 6).

Figure 6. Wyatt DAWN instrument dashboard display indicating a ready status.

III. COLUMN USE CONSIDERATIONS

A. MOBILE PHASE PREPARATION GUIDANCE

Only use high quality, filtered water (*i.e.*, Milli-Q Millipak® 0.22 µm filtered water) when preparing SEC mobile phases. For SEC optical detection experiments, it is advice to filter mobile phases with a <0.22 µm filter. Sterile units containing 0.2 µm nylon filters have been successfully used for this purpose (*i.e.*, Fisher Scientific catalog no. 09-740-46). Solutions that are susceptible to microbial growth (*e.g.*, SEC eluents that do not contain an antimicrobial such as sodium azide, methanol, isopropanol, or acetonitrile) should be replaced at regular 2–3 day intervals to avoid column contamination. The use of a bactericidal additive is strongly recommended, whenever possible. Do NOT refill partially full SEC reservoir bottles with new mobile phase buffer, as this can lead to the rapid propagation of microbial organisms in the fresh mobile phase. Rather, use a new bottle containing the freshly prepared SEC buffer. Solvent inlet filters (sinker filters) are often a source of bacterial contamination, and it is highly recommended that they not be used. It is also recommended to avoid the use of silica-based sintered glass filter supports when filtering mobile phases of pH > 6.8 due to the potential of introducing soluble and/or insoluble silicates into the SEC mobile phase which can potentially alter column performance. Lastly, it is recommended to perform periodic (weekly) flushes of 70% alcohol solution on your LC lines to ensure contamination from bacterial growth is kept in check.

B. BUFFER SELECTION

GTxResolve Premier SEC 1000 Å SEC Columns are designed to provide industry-leading method flexibility and are robust against a wide range of buffers. In many cases, a previously developed method may be used to achieve exceptional performance without further optimization. However, the inertness of these columns facilitates the use of reduced buffer and salt. A 10-20 mM concentration of buffer reagent with a 276 mM NaCl, and 5.4 mM added concentration of potassium chloride, pH 7.0-7.4, is a good starting point as mobile phase. Phosphate buffered saline (PBS) can also be applied for many general-purpose applications, and it is a simple to prepare solution given the availability of PBS packets and tablets. Regardless of preparation, it is critical to filter all buffers through a 0.2 µm filter or 0.1 µm filter for light scattering (LS) to reduce the risk of particulate and/or microbial fouling of the column. The use of a bactericidal additive is also recommended.

Organic co-solvents are commonly used in SEC mobile phases to mitigate hydrophobic secondary interactions. If an organic modifier is employed, methanol, ethanol, acetonitrile, and isopropanol (IPA) are recommended for use with these columns. It is advised to use up to 10% organic in the mobile phase and to increase that concentration only if necessary, up to a maximum of 15%. Higher concentrations of organic co-solvent can lead to partial denaturation of biomolecules. That said, the column is compatible with much higher organic concentrations if a particular type of analysis warrants the use of high levels of organic solvent (e.g. up to 50% organic).

C. COLUMN INSTALLATION FOR GENERAL PURPOSE APPLICATIONS

Prior to placing the column on the system, purge the system of any organic or water-immiscible mobile phases. Purge the entire fluidic path through the detector to the waste container. When connecting the column inlet, orient it in the proper direction as noted by the arrow on the column endnut.

Check that ferrules are adjusted to the optimal depth to ensure a low dead volume connection. GTxResolve Premier SEC 1000 Å SEC Columns are shipped in a solution containing 10% acetonitrile/90% 25 mM sodium phosphate pH 7.0 + 100 mM KCl. Flush column to waste with filtered buffer using no less than 20 column volumes. Equilibrate instrument with buffer and ensure MALS noise level is acceptable before connecting column.

Equilibrate the column at desired flow rate in your buffe. Increase flow rate slowly in 0.1 mL/min increments to purge the column for a minimum of 10 column volumes (refer to Table 2 for column volumes and pressure limits). Monitor system pressure to ensure that a stable pressure trace is achieved. This will confirm the readiness of both the column and your LC pump for your method runs.

Table 2. Column Volumes, Pressure Limits, and Max Recommended Flow Rates

The lower the flow rate, the better the SEC resolution. 0.1-0.25 mL/min flow rate gives best result for 4.6 mm column and the linear velocity may be proportionately increased for larger diameter (7.8 mm) columns. Faster flow rates can lower the resolution performance of closely eluting peaks. Maximum flow rates for each column dimension are given below.

Column Dimension	Approximate Volume	Column Pressure Drop Limit	Max Recommended Flow Rate
4.6 x 150 mm	2.5 mL	≤ 2300 psi (160 bar)	≤ 1.0 mL/min
4.6 x 300 mm	5.0 mL	≤ 4600 psi (320 bar)	≤ 1.0 mL/min
7.8 x 150 mm	7.0 mL	≤ 2300 psi (160 bar)	≤ 1.5 mL/min
7.8 x 300 mm	14.0 mL	≤ 3750 psi (320 bar)	≤ 1.5 mL/min

The maximum pressure limits above are for the pressure drop across the column. If columns are operated at conditions above these guidelines, column lifetime will be negatively affected.

*The Maximum recommended pressure drop across the column refers only to the column itself and NOT from the added pressure contribution from the LC System.

To determine the pressure drop across the column: install a zero dead volume union in place of the column and determine the system pressure at operating conditions, and subtract that value from the system back pressure observed with the column installed. For best column lifetime, flow rates should be chosen so as not to exceed the max column back pressure. Lower flow rates will generally provide high resolution separations (at the compromise of run time). The maximum recommended pressure drop across the column is provided as a guideline to ensure longest possible column lifetimes.

D. CONFIRMING COLUMN PERFORMANCE

Upon receiving a new column and periodically throughout its lifetime, Waters recommends measuring and tracking column efficiency. A uracil separation can be performed.

A 0.1 mg/mL solution of uracil prepared in your buffer can be used to benchmark the column's performance by performing an injection using the conditions given on the Column Test Report. The Column Test Report contains uracil retention time, 5-Sigma efficiency, USP tailing, and column pressure data. Your results may vary based upon system dispersion.

A deleteriously low uracil efficiency (plate count) will indicate that the column has become mechanically compromised. Often, it would take more than a 20–30% drop in uracil plate count before an AAV separation is seen to be negatively affected.

E. INJECTION VOLUME AND MASS LOAD RECOMMENDATIONS

Recommended injection volumes and Mass loads are provided in Table 3. Larger injection volumes can be used but may result in slightly lower resolution between the monomer and HMWS. Note: when only small amounts of sample are present in a vial, it is recommended to set needle depth to 1 mm from the bottom of the vial. If injections lead to blank chromatograms, check the needle depth.

Table 3. Injection Volume and Mass load Recommendations

Diameter (mm)	Length (mm)	Injection Volume (µL)	Mass Load
4.6	150	<10 µL	<100 µg
	300	<20 µL	<200 µg
7.8	150	<30 µL	<300 µg
	300	<60 µL	<600 µg

Note: The injection volumes and mass loads listed above are reflective of legacy methods on columns and systems that suffered from substantial and prolonged sample losses. Today, much lower injection volumes and mass loads provide better resolution for harder to resolve peaks. This can be confirmed for well resolved peaks by making a series of increasing injection volumes and obtaining equivalent characterization data.

F. 4.6 X 30 MM GUARDS FOR BOTH 4.6 AND 7.8 MM ID ANALYTICAL COLUMNS

Use of the specially developed GTxResolve Premier SEC 1000 Å, 4.6 x 30 mm Guard can effectively prolong analytical column lifetimes. The useful lifetime of the guard can be influenced by many factors including: mobile phase cleanliness/microbial contamination, sample precipitates/aggregation, excipients in sample formulations, and working at extremes of pressure, pH, and/or temperature. Guard columns may need replacement if significant increases in column pressure and wide tailing or split peaks are observed. The GTxResolve Premier SEC 1000 Å, 3 µm 4.6x30 mm Guard is a low dispersion device and it has been optimized for use on both 4.6 and 7.8 mm ID analytical columns.

Injection of particulates as well as excipients contained in many sample matrices may also shorten its useful life. Consequently, it is important to ensure samples are free of particulates before injecting them onto the SEC column.

If samples appear cloudy or turbid, they should not be injected, as this could lead to column pressure increases. Sample preparation such as a 0.2 or 0.45 µm syringe filtration or centrifugation may be used, if appropriate.

G. DETECTOR FLOW CELLS (SPECIAL NOTE)

Waters standard analytical flow cells contain PTFE wetted parts that can produce tailing peaks and incomplete recovery for biomolecules, most especially when performing aqueous SEC. When possible, always select Bio-inert or Bio-compatible parts for your systems. Titanium flow cell is available from Waters for aqueous bioseparations and is highly recommended for use with biologics including LNPs, AAVs, antibodies, etc.

Recommended Waters Flow Cells:

ACQUITY Premier

TUV:	205000611	ACQUITY UPLC TUV 5mm Titanium FC
PDA:	205000613	ACQUITY UPLC PDA 5mm Titanium FC
FLR:	205002267	ACQUITY Premier FLR Flow Cell Arc Premier
TUV:	205001731	Low Dispersion, Bio
PDA:	205001043	Analytical Inert TS
FLR:	205002279	Low Dispersion Analytical

IV. COLUMN SPECIFICATIONS

- Shipping solvent: 10% acetonitrile/90% 25 mM sodium phosphate pH 7.0 + 100 mM KCl.
- pH range: 2.5–8.5
- Temperature range: 4–50 °C
- Recommended salt concentration: 100–500 mM KCl or NaCl
- Recommended buffer concentration: 10–100 mM
- Organic concentration: <10% isopropanol, <15% acetonitrile

V. INTACT LNP METHOD CONSIDERATIONS (SPECIAL NOTE):

LNP's may exhibit some solubility issues in fully aqueous conditions thus resulting in clogging of flow path, LC sample injector in addition to column fouling. In order to keep the residual lipid component or disrupted LNP from adsorbing to the packing material and fouling the column, a mobile phase mixture of 2x strength PBS, 5% IPA and 0.001% Pluronic F- 68 can be used as starting point. Additionally, it is recommended to dilute the sample with 5%IPA / 95% 2x PBS to improve its solubility and until the haziness of a turbid sample disappears.

A relatively low flow rate (of 0.1 mL/min) is recommended for use with 4.6 x 150 mm column. Methods can be scaled to increased flow rates and longer columns after confirming successful runs a column temperature of 30°C is also recommended. Maintain the sample manager temperature at 5 °C and do not use sample for > 24hr once vial cap has been perforated. Poor recovery of LNP and severe amounts of peak tailing can be enhanced using low adsorption hardware detector flow cell. Because there are many varying types of LNP formulations, it is recommended to use a 4.6 x 30 mm guard column for all intact LNP analyses.

VI. TROUBLESHOOTING

The first step in systematic troubleshooting is comparing the column performance in its current state to the performance when it was functioning properly. There are several common symptoms of change in the column.

1. An increase in pressure is often associated with decreased performance in the application. The first step in diagnosis is to ensure that the elevated pressure resides in the column rather than somewhere else in the system. This is determined by monitoring system pressure as each connection is broken from the outlet end to the inlet. If the system is occluded, the blockage should be identified and removed. If the pressure increase resides in the column, it is helpful to know whether the problem was associated with a single injection or if it occurred over a series of injections. If the pressure gradually builds up, it is likely that the column can be cleaned as described in Section VI. If a single sample caused the pressure increase, it likely reflects particulates or insoluble components, such as lipids or higher order insoluble aggregates. Cleaning is still an option but using the more aggressive options. If samples appear cloudy or turbid, they should not be injected, as this will lead to pressure increases. Sample preparation such as filtration or centrifugation may be used, but one should first check whether this impacts the results.
2. Loss of resolution and increased peak tailing can be caused by microbial contamination. It is important to follow good standard laboratory practices to prevent microbial contamination. This includes changing buffer bottles frequently, using high purity water, using a sterile filtration apparatus, and storing system and column under recommended conditions. If microbial contamination has occurred, cleaning the column will have no effect on performance.
3. Increased peak tailing can be caused by failure of a tubing connector or a buildup of material on the column inlet frit. Before proceeding with diagnostic or corrective measures, check all connections, that the mobile phases have been correctly prepared, and the correct method has been selected. If the increased peak tailing is again observed, it is likely that there is significant buildup of material on the column inlet and the column will require replacement.
4. Carryover is defined as the appearance of the constituents of one sample in the next analysis. In size-exclusion chromatography, carryover is typically due to system components or improper wash solvents. Run a blank injection. If the protein peaks only appear when an injection is made, they likely originated from system component or inadequate wash solvents. Adsorption on system components most likely occurs in the loop or needle. In these instances, the component may need to be changed.

VII. COLUMN CLEANING AND STORAGE

A. COLUMN CLEANING

Flush the column at one half the normal flow rate. One or more of the following may be used:

- 25–200 mM buffer solution, pH 3.0 + 0.5 M NaCl or KCl
- 10% acetonitrile or 20% methanol combined with 25–100 mM buffer + 100 mM NaCl or KCl (do not exceed 20% organic)
- Do not use ionic detergents and other surfactants
- Reversal or back flushing can be tried, but may further damage the column, or only provide short lived improvement in column performance.

B. COLUMN STORAGE

Storage of the column in 100% water or 100% buffer is not recommended since this may compromise column performance and allow microbial growth. The recommended storage solution is to purge the column with the shipping solvent (10% Acetonitrile/90% 25 mM sodium phosphate pH 7.0 + 100 mM KCl). A minimum of ten-column volumes should be used. When storing the column in the shipping solvent listed above, it is preferable to keep the column at ambient (room) temperature.

VIII. COLUMN QR CODE

The quick reference (QR) code that is located on the column label provides column-specific information (i.e., the part and serial numbers that are unique identifiers for the column), and its encoding follows a widely adopted industry-standard.

1. Scan QR code using your preferred device.
2. Be directed to the column's information hub on waters.com.
3. Access technical and scientific information for the column (i.e., certificate of analysis, application notes).

IX. CAUTIONARY NOTE

Depending on their final application, these products may be classified as hazardous following their use, and as such are intended to be used by professional laboratory personnel trained in the competent handling of such materials. Responsibility for the safe use and disposal of products rests entirely with the purchaser and user. The Safety Data Sheet (SDS) for this product is available at Waters.com/sds. Research only. Not for IVD use.

Part Number	Column
186010733	GTxResolve Premier SEC Guard, 1000Å, 3 µm, 4.6x30 mm 1/pk (for use with both 4.6 and 7.8 mm ID analytical columns)
186010735	GTxResolve Premier SEC 1000Å, 3 µm, 4.6x150 mm
186010736	GTxResolve Premier SEC 1000Å, 3 µm, 4.6x300 mm
186010737	GTxResolve Premier SEC 1000Å, 3 µm, 7.8x150 mm
186010738	GTxResolve Premier SEC 1000Å, 3 µm, 7.8x300 mm
186006842	Waters Protein Standard Mixture
186010778	Waters dsDNA 50 to 1350 ladder

References

1. Guide to Size-Exclusion Chromatography (SEC) March 2020. Literature number 720006067EN.
2. Guidelines for Improving the Quality of SEC MALS Data in an Aqueous System.
3. SEC MALS Noise Assessment and Cleaning Guide.

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