APPLICATION NOTE

Advanced Size-Exclusion Chromatography for Lentivirus Aggregation Analysis

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Characterization of Lentivirus Aggregation using Sepax SRT-LVL SEC 2000 Å

Key Features

- **Ultra-wide Pore SRT-LVL SEC (2000 Å):** Enables size-based separation of Lentivirus from larger aggregates and smaller impurities.
- **Hydrophilic Coated Surface:** Minimizes nonspecific interaction, resulting in improved recovery, enhanced peak symmetry, and more consistent size-based separation.
- **High Pore Volume:** Achieves exceptional resolution for robust aggregate profiling and the streamlined separation of large molecules.
- **Low Noise and System Compatible:** Suitable for native conditions and flexible detection (e.g., UV, FLD, SEC-MALS) for robust aggregation analysis.

Lentiviruses are a subgroup of retroviruses in the family Retroviridae that can infect both dividing and non-dividing cells, enabling their broad use as gene delivery vectors in research, biomanufacturing, and gene and cell therapy. Modern lentiviral vectors are engineered from HIV-1 by removing pathogenic and replication-competent genes and replacing them with therapeutic or reporter transgenes to ensure an improved safety profile.

Structurally, lentiviruses are large, enveloped particles ($\sim$ 80–120 nm, up to $\sim$ 150 nm for elongated forms) surrounded by a host-derived lipid membrane bearing viral glycoproteins such as Env or VSV-G, which govern cell entry and tropism. The viral core contains a conical capsid enclosing a $\sim$ 9 kb single-stranded RNA genome together with essential enzymes, including reverse transcriptase, integrase, and protease.

Due to their large size, complex envelope, and intrinsic heterogeneity, lentiviral vectors are prone to aggregation during production, purification, formulation, and storage. Robust analytical methods are essential for distinguishing intact particles from aggregates and degradation products.

Aggregation analysis using an ultra-wide pore SRT-LVL SEC column provides a size-based, non-denatured approach for Lentivirus characterization. The pore size of 2000 Å accommodates lentiviral particles with minimal nonspecific interactions, and enables absolute determination of particle size and aggregation state, supporting process development, formulation optimization, and stability assessment.

Technical Specifications

Phase	SRT-LVL SEC
Particle size	5 μm
Pore size	2000 Å
Silica	spherical, high purity
Surface	chemically bonded monolayer
Recommended Sample Types	water-soluble polymers, viral particles, long-chain oligonucleotides, and lipid nanoparticles

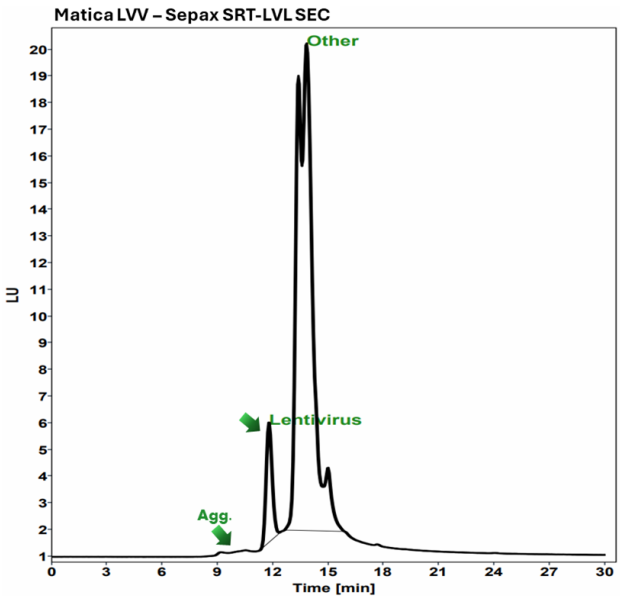
HPLC Method	Details
Mobile Phase	80 mM phosphate buffer, 50 mM KCl, 250 ppm NaN_3, pH 6.5
Flow Rate	0.3 mL/min
Detection UV	UV 260 and 280 nm
Detection FLD	Ex=284 nm and Em=345 nm
Instrument	Agilent 1260 Infinity II HPLC

Sample	Lentivirus
Sample Info	Lentivirus
Sample Vendor	Matica Biotechnology
DLS	Hydrodynamic radius: 80 nm PDI: 0.27

Results and Conclusion

The Sepax SRT-LVL SEC column is particularly suitable for lentivirus aggregation analysis because its ultra-wide pore (2000 Å) architecture ensures virus-sized particles are resolved via size-exclusion mechanisms without pore occlusion. The hydrophilic surface effectively suppresses nonspecific interaction between the lentiviral envelope and the stationary phase, which is critical for achieving high recovery and accurate aggregate quantification. In addition, the high pore volume and stable bonded coating of the phase support reproducible separations under fully aqueous, native buffer conditions and ensure compatibility with SEC-MALS detection, enabling reliable, absolute characterization of intact lentiviral particles and aggregates across development and manufacturing workflows.

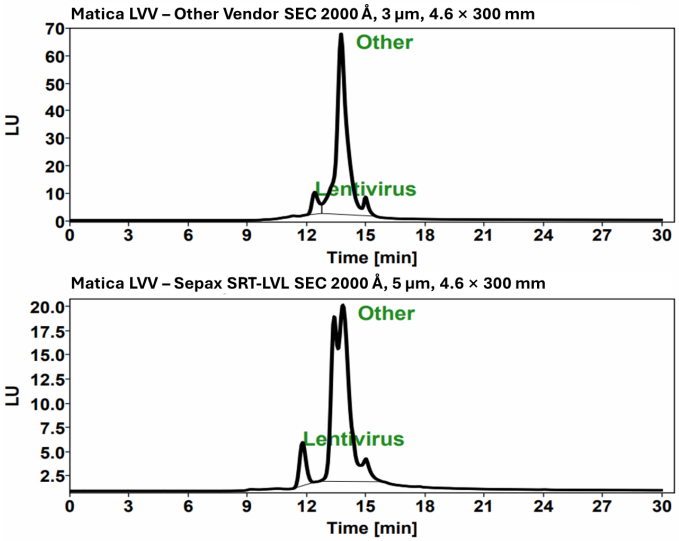
Figure 1. Aggregation Analysis of Matica Lentivirus on the Sepax SRT-LVL SEC 2000 Å column.



► Sepax Ultra-wide Pore SRT-LVL SEC

A key differentiator of the Sepax SRT-LVL SEC phase is its chemically bonded, neutral hydrophilic monolayer. This specialized surface is engineered to minimize the secondary interactions typically encountered with lipid-, PEG-, and polysaccharide-conjugated biomolecules. The special coating effectively shields the underlying silica surface, minimizing nonspecific binding that commonly occurs with enveloped, inherently "sticky" viral particles. In addition, this engineered surface optimizes resolution and peak capacity by ensuring that separation is governed strictly by size exclusion, preventing unintended secondary interactions from degrading chromatographic performance. Together, these properties significantly improve recovery of intact lentiviral particles, suppress artificial peak broadening or tailing, and enable cleaner, more reliable aggregation profiles. As a result, the SRT-LVL SEC phase delivers robust size-based separations for lentiviral vectors and other lipid-, PEG-associated biotherapeutics.

Figure 2. Evaluating the Resolution of Lentiviral Vectors Using Sepax SRT-LVL and Competitor 2000 Å Columns with dimensions of 4.6 × 300 mm.

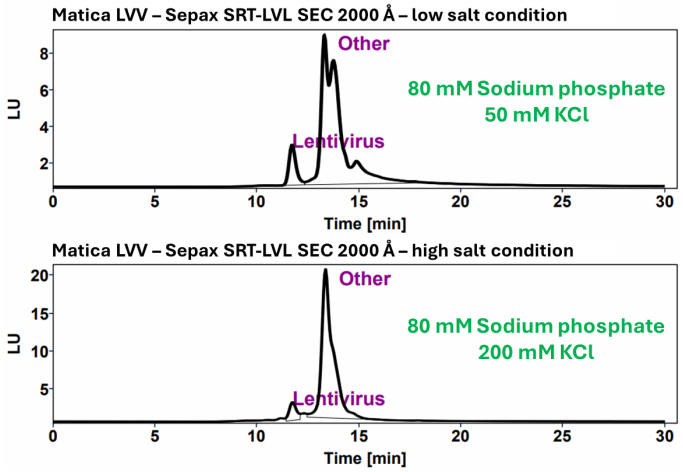


In comparative evaluations, the Sepax SRT-LVL SEC column outperforms competing SEC phases, particularly for lentiviral vectors, as shown in Figure 2. The engineered hydrophilic monolayer of the SRT-LVL SEC stationary phase effectively suppresses undesirable secondary interactions between the modified surfaces of the analytes, such as viral envelopes, and the silica base. This minimization of non-specific interaction results in higher mass recovery, improved peak symmetry, and superior resolution between targeted particles, their aggregates, and associated impurity. By contrast, less hydrophilic surfaces frequently exhibit increased non-specific binding/ interaction, which induces peak broadening and deviates from a pure size-exclusion mechanism, ultimately compromising the capacity and accuracy of aggregate quantitation.

► Method Optimization

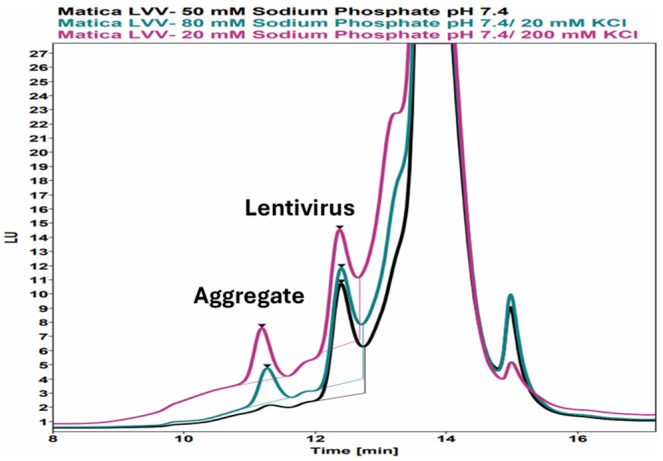
The SRT-LVL SEC phase is fully compatible with the aqueous buffer systems standard in upstream and downstream bioprocessing, including phosphate, Tris, and volatile ammonium acetate mobile phases. This versatility enables characterization under non-denaturing conditions, preserving the structural integrity of viral vectors during analysis. As with most SEC-based viral characterization, method optimization, specifically the fine-tuning of salt and ionic strength, is essential to maximize resolution across diverse size regimes and ensure the accurate quantitation of both monomeric species and high molecular weight (HMW) aggregates.

Figure 3. Impact of Salt Concentration on Resolution and Aggregation Profiling of Lentiviral Vectors in SEC Analysis.



As shown in Figure 3, adjusting salt concentration and overall ionic strength significantly influences the column behavior. Moderate increases in ionic strength reduce residual electrostatic interactions between the lentiviral envelope, aggregates, and the stationary phase, leading to improved peak shape and enhanced signal sensitivity between HMW aggregates and targeted lentivirus.

Figure 4. Impact of Ionic Strength on Signal Sensitivity and Aggregation Profiling of Lentiviral Vectors in SEC Analysis.



However, at excessively high salt concentrations (e.g., elevated KCl, shown in Figure 4), the resolutions of lower molecular weight (LMW) species are reduced. This effect arises because strong ionic shielding suppresses not only undesirable secondary interactions but also subtle size-dependent retention differences among smaller species, reducing their resolution. In addition, high salt levels can decrease effective pore accessibility for LMW components and increase mass-transfer limitations, further contributing to peak coalescence.

Together, these observations highlight the importance of optimizing ionic strength to achieve a balanced separation, maximizing aggregate and the targeted virus resolution while preserving sufficient selectivity for LMW impurities.

Moreover, the low-noise baseline and minimal particle shedding of the SRT-LVL SEC phase also make it ideally suited for SEC-MALS workflows. The integration of UV and Multi-Angle Light Scattering (MALS) detectors enables the accurate characterization of particle molar mass and size, providing a better assessment of the aggregation state.

Summary

- SRT-LVL SEC for viral-sized particles: 2000 Å pore size is designed to accommodate very large biomacromolecules, enabling size-based separation of targeted virus particles vs. HMW aggregates and LMW impurities/fragments.
- Engineered hydrophilic Surface minimizes nonspecific binding: The stationary phase uses a hydrophilic coating that helps shield the silica surface and reduce undesired interactions, important for "sticky" viral vectors, supporting higher recovery and cleaner aggregate profiles.
- High pore volume and separation capacity: The SRT-LVL SEC resins are described as having a specially designed ultra-wide pore volume, which translates to higher loading capacity and improved resolution for large biomolecules/particles.
- Detector flexibility: The SRT-LVL SEC phase is fully compatible with UV, MALS, and other detections, delivering low baseline noise and stable signals without requiring extended equilibration times, thereby enabling efficient and reliable aggregation characterization.

Order Information

Phase	Pore Size	Part Number	Column Dimension
SRT-LVL SEC	2000 Å	219980-7830	7.8 × 300 mm
SRT-LVL SEC	2000 Å	219980-7805	7.8 × 50 mm, guard column
SRT-LVL SEC	2000 Å	219980-4630	4.6 × 300 mm
SRT-LVL SEC	2000 Å	219980-4605	4.6 × 50 mm, guard column