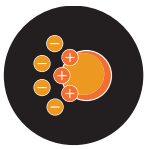
Evaluation of Lentiviral Vector Stability and Development of an Ion Exchange Purification Process



ANION EXCHANGE

Gene therapy vectors such as lentiviral vectors (LVVs) continue to be of great therapeutic importance for a variety of biopharma disease targets. A challenge for process-scale purification of LVVs is achieving purity while maintaining the stability of the LVVs. Ion exchange chromatography (IEX or IEC) is a powerful method for purifying LVVs, and specific IEX resins can be advantageous in particular workflows. For example, Macro-Prep™ High Q Resin, a strong anion exchange resin, offers numerous advantages for purifying LVVs. Its large particle size provides high flow rates and allows for easy scale-up, and the resin has a high binding capacity, ensuring efficient purification even at large scales. In *Evaluation of lentiviral vector stability and development of ion exchange purification processes*, Ghosh et al. (2022), at Rensselaer Polytechnic Institute, examined the stability of LVVs and subsequently developed ion exchange–based purification processes for LVVs. In this article, we present key findings of their research showing that Macro-Prep High Q Resin is an effective choice for LVV purification.

Workflow and Results

Ghosh et al. investigated the stability of LVVs under various conditions, including pH, temperature, and ionic strength. They determined that the vector exhibits good stability in phosphate buffer at pH 6.5–7.5, with low to moderate salt concentrations. These findings were then used to select conditions for screening various resins for LVV purity and integrity. A high-throughput batch screen was carried out under stable conditions to identify optimal wash and elution steps to improve product yield and protein clearance. Next, linear gradient experiments were conducted in a mini-column format to refine the operating conditions. Final step gradient processes were established that exhibit greater than 70% yield of infectious LVVs while also achieving a reduction of host cell proteins (HCP) during the process.

IEX Resin Screening for LVV Recovery

A high-throughput process development (HTPD) approach was used for designing the LVV purification process. For the first screening phase, candidate resins were screened on a 96-well plate (see Appendix A, Table 1A for suppliers). This slurry plate technique (Figure 1) allowed for broad resin screening with the primary goal of identifying resins that provided good lentivirus recovery measured using reverse transcription quantitative PCR (RT-qPCR).

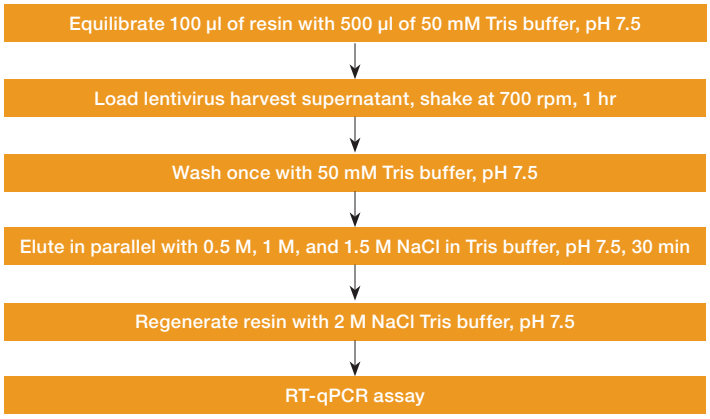


Fig. 1. Slurry plate workflow. For each test, 100 µl of a candidate resin was added to a 96-well membrane-bottom vacuum plate well and equilibrated with 500 µl of 50 mM Tris buffer, pH 7.5 (equilibration buffer, 5:1 mobile to solid phase volume ratio). Following equilibration, the lentivirus harvest supernatant was incubated and shaken for 1 hr. Next, unbound impurities were removed by washing with the equilibration buffer. In the elution step, one of three different salt concentrations was applied to elute the lentivirus: 0.5, 1.0, and 1.5 M NaCl in 50 mM Tris buffer, pH 7.5. The experiments with the different salt concentrations were performed in parallel (a single salt concentration was used for each elution step). Finally, resins were regenerated using 2.0 M NaCl buffer, and the eluted fractions were analyzed by reverse transcription quantitative PCR (RT-qPCR) to compare the LVV recovery yield.

A comparison of the lentivirus yield percentage from the various resins used in the HTPD slurry plate screen is shown in Figure 2. Of the 26 chromatographic resins screened, the authors observed that only 11, including Macro-Prep High Q, resulted in greater than 50% LLV recovery. Thus, Macro-Prep High Q Resin was selected for the next level of high-throughput screening. Additionally, Macro-Prep High Q Resin was one of only eight resins that delivered an LVV recovery of more than 60% when eluted with 1.5 M NaCl.

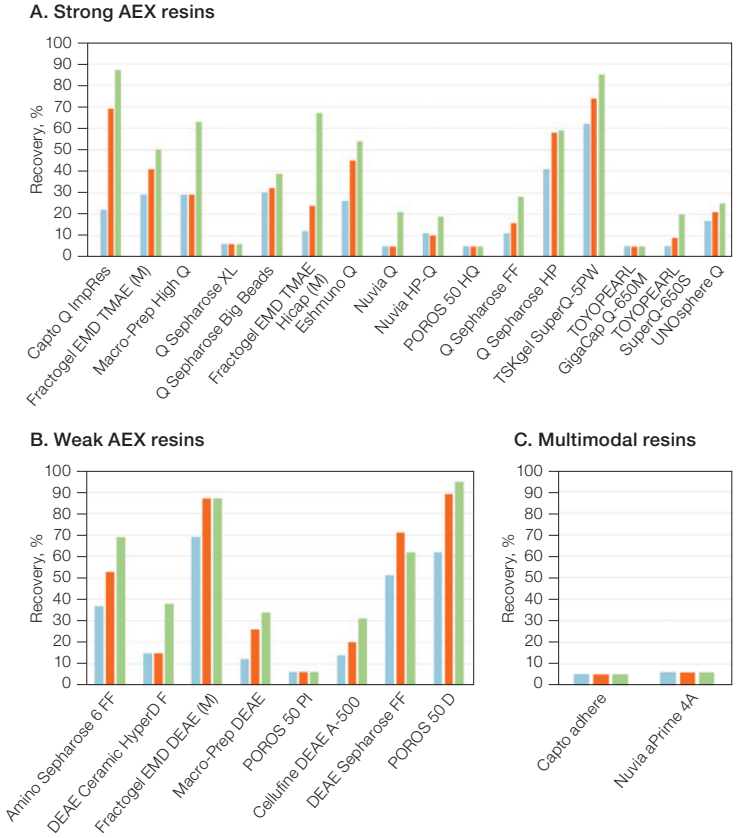


Fig. 2. Elution recovery of lentivirus from batch screening of chromatographic resins using parallel elution conditions. Recovery for **A**, strong AEX resins, **B**, weak AEX resins, and **C**, multimodal resins with elution conditions of 0.5 M NaCl (■); 1.0 M NaCl (■); 1.5 M NaCl (■). LVV titer was analyzed using RT-qPCR assays in duplicates. AEX, anion exchange chromatography; FF, Fast Flow; HP, High Performance.

IEX Resin Screening for Resolution and Selectivity

For the second screening phase, top performing anion exchange (AEX) resins, shown in Table 1, were further tested for process conditions by altering the pH and salt concentration of the buffer. This second screening study aimed to evaluate the resins' resolution and selectivity, that is, how well a high-yield candidate resin separated the lentivirus from other impurities, such as host cell proteins. Lentivirus stability data were also taken into consideration for this set of screening. The authors found that phosphate buffer was most stabilizing compared to other buffering salts such as Bis-Tris propane, MOPS, MES, etc. Hence, further experimentation was carried out with phosphate buffer.

Table 1. Resins tested in the secondary screen for resolution and selectivity.

Top-Performing AEX Resins
Capto Q ImpRes
Amino Sepharose 6 Fast Flow
Fractogel EMD DEAE (M)
Fractogel EMD TMAE (M)
Macro-Prep High Q
DEAE Sepharose Fast Flow
Fractogel EMD TMAE Hicap M
Q Sepharose High Performance
TSKgel Super Q5-PW
Eshmuno Q
POROS 50 D

AEX, anion exchange chromatography.

The process-condition screening was carried out using the same method as before with the 11 resins (Table 1) that showed promising LVV recovery, except that the elution conditions were evaluated by varying the pH and salt concentrations of the buffer. The results for Macro-Prep High Q Resin are shown in Figure 3.

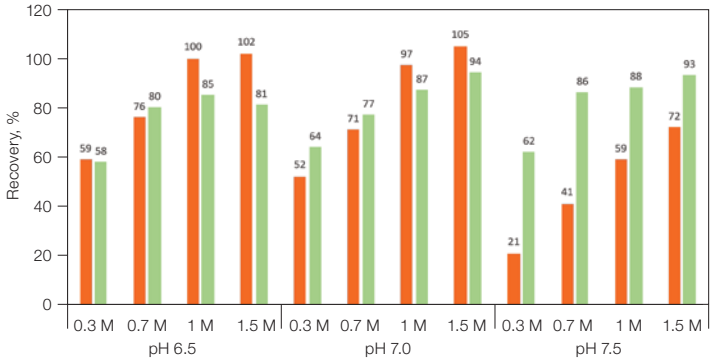


Fig. 3. Batch elution data for Macro-Prep High Q Resin. Using the 96-well-plate batch screening format, pH was evaluated at three different pH conditions: pH 6.5, pH 7.0, and pH 7.5. The salt concentration was evaluated for each pH at four conditions: 0.3 M, 0.7 M, 1.0 M, and 1.5 M NaCl. The resulting elution fractions were then analyzed by RT-qPCR in duplicate to determine the % LVV recovery in terms of the physical titer (■). The residual protein impurities (■) were analyzed using the Quick Start Bradford Protein Assay Kit from Bio-Rad.

Results from this second screen further support that Macro-Prep High Q Resin can be effective for LVV purification. Figure 3 shows LLV recovery with respect to changes in pH and salt concentration. The authors found that the purity and recovery of the LVVs were influenced by the pH and ionic strength of the binding and elution buffers; a decrease in elution pH increased lentiviral vector recovery.

This behavior has important implications for process design: wherein a significant fraction of the protein impurities could potentially be washed from the column at a lower salt concentration. At the same time, the lentivirus could then be eluted at a higher salt condition at a lower pH of 6.5. Eluting at a lower pH also implied that a lower salt elution could be employed, which, in turn, can enhance the lentiviral stability, since high salt conditions are detrimental to lentivirus stability. These observations

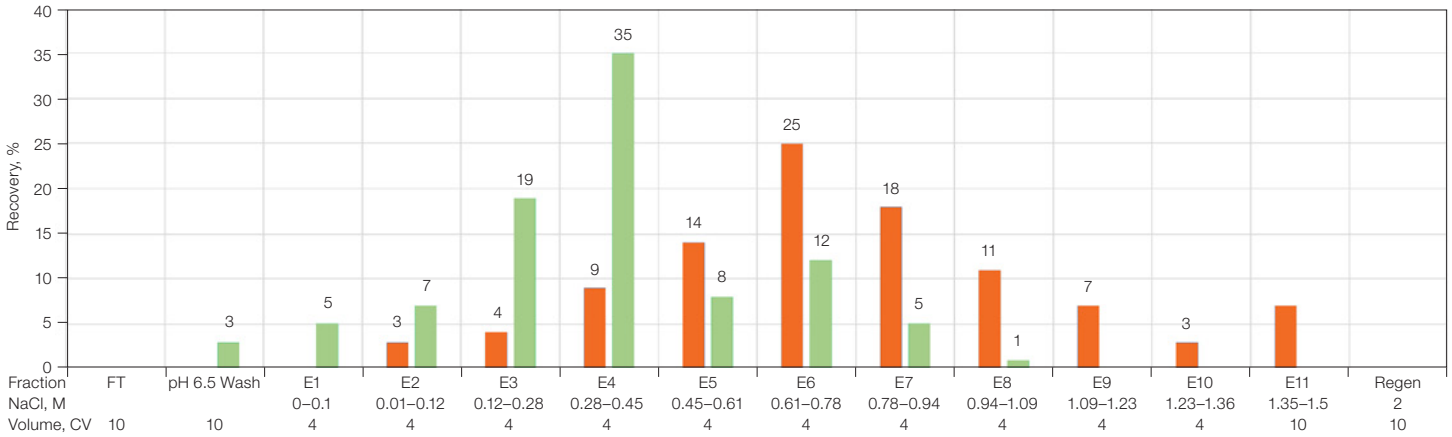


Fig. 4. LVV elution recovery and protein clearance for linear gradient experiments with Macro-Prep High Q Resin. LVV titer (■) and protein amount (■) in respective fractions were measured in duplicates using RT-qPCR and Bradford assay, respectively. The pool volume in each fraction is represented in column volume (CV), and elution salt concentrations corresponding to eluate fractions are indicated in the figure. FT, flowthrough; Regen, resin regeneration.

and the resin's other properties make it optimal for process-scale workflows and supports screening Macro-Prep High Q Resin when developing lentiviral vector purification methods.

Scale-Up of LVV Purification Using Marco-Prep High Q Resin and a Linear Gradient

To confirm what was observed from batch screening, Ghosh et al. further evaluated the resolution and selectivity of the Macro-Prep High Q Resin by scaling up to a 0.5 ml mini-column format and the established process conditions that provided high LVV recovery and good impurity clearance. A linear gradient from 0–1.5 M NaCl was applied for eluting the lentivirus. Column volume (CV) elution fractions were collected during the gradient and were analyzed by RT-qPCR and Bradford Assay.

As shown in Figure 4, protein impurities required a lower salt concentration for elution, whereas the LVVs required a relatively higher salt concentration. These data further confirmed that Macro-Prep High Q Resin can also be utilized for lentivirus purification in dynamic column-based conditions.

Scale-Up of LVV Purification Using Marco-Prep High Q Resin and a Step Gradient

As a final study based on the results of the screening studies and the mini-column experiments, the authors finally devised a step gradient elution process for LVV purification. The process outputs are presented in Figure 5. The LVVs were successfully separated from the protein impurities using a salt step gradient and resulted in a 78% LVV physical titer recovery based on RT-qPCR data, with an 85% protein impurity clearance (Table 2).

As shown in Table 2, the RT-qPCR, p24 ELISA, and transduction infectivity assay results are consistent, confirming that high recoveries were achieved with the step gradient process and that the lentivirus particles maintained their infectivity during the process. The fivefold dilution of lentiviral eluate fractions to reduce the salt concentration in the final product pool, and the relatively short exposure time of the lentivirus to the elevated elution salt conditions in the column, were both likely beneficial to maintaining infectivity. Additionally, the HCP ELISA assay showed a greater than 2.5 log reduction in the HCPs. In comparison to three other

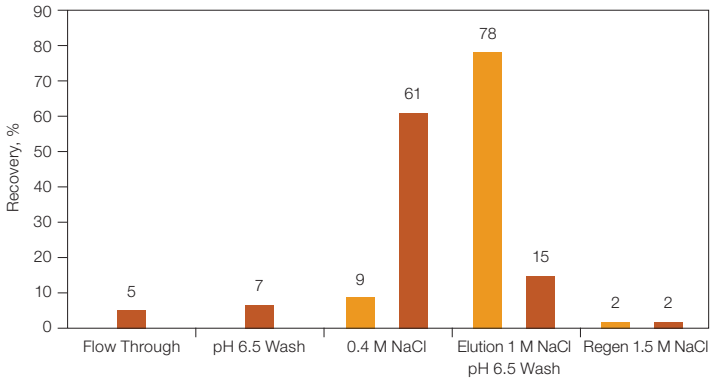


Fig. 5. LVV elution recovery and protein clearance for step elution with Macro-Prep High Q Resin. Chromatography steps shown with LVV titer (■) and protein amount (■) measured in duplicates using RT-qPCR and Bradford assay, respectively. The pool volume in all fractions is 10 CV. Regen, resin regeneration.

Table 2. Results of the optimized step elution process indicating LVV recovery and HCP clearance achieved with Macro-Prep High Q Resin. LVV recovery was measured using orthogonal techniques: total viral RNA by RT-qPCR, capsid p24 protein content by p24 enzyme-linked immunosorbent assay (ELISA), and infectivity by a transduction assay. An ELISA for residual HCP (HEK 293) was also performed.

	LVV recovery, %			HCP clearance, LRV
Resin	**RT-qPCR**	**p24 ELISA**	**Infectivity**	**HEK 293 HCP ELISA**
Macro-Prep High Q	78	86	82	2.51

HCP, host cell protein; HEK, human embryonic kidney; LRV, logarithmic removal value.

resins (see Ghosh et al. 2022), which were also screened and tested using the same assays, the final optimized process utilizing Macro-Prep High Q Resin resulted in the most significant HCP reduction. The optimized conditions provided by Ghosh et al. therefore serve as a starting point for process development using Macro-Prep High Q Resin.

Application of Macro-Prep High Q Resin

Macro-Prep High Q Resin, a strong anion exchange resin, offers numerous advantages for the purification of LVVs. Its large particle size provides high flow rates and allows for easy scale-up. The resin also has a high binding capacity, ensuring efficient purification even

at large scales. Macro-Prep High Q Resin is chemically stable and compatible with a wide range of buffers and solvents commonly used in biopharmaceutical manufacturing. Macro-Prep High Q Resin's scalability allows for efficient purification for biopharma manufacturing workflows. Moreover, the resin can withstand pH 1–10 environments and can retain full functional performance in the presence of acid and detergent treatment. This is due to its properties, such as rigid methacrylate matrix, which provides superior mechanical, thermal, and chemical stability. Macro-Prep High Q Resin is a slightly hydrophobic base bead that has high ligand density and is available with an average particle size of 50 µm. Macro-Prep derivatives and functionalities include Q and S types (strong IEX resins), diethyl aminoethyl (DEAE) and carboxymethyl (CM) types (weak AEX resins), and methyl and t-butyl HIC types.

Conclusions

The studies conducted by Ghosh et al. highlight the importance of evaluating LVV stability and developing robust purification processes. Macro-Prep High Q Resin proved to be a reliable and efficient tool for the purification of LVVs, providing high purity and excellent recovery. This resin can be an excellent choice for researchers and biopharmaceutical manufacturers seeking to optimize their purification processes for LVVs.

References

Ghosh R et al. (2022). Evaluation of lentiviral vector stability and development of ion exchange purification processes. Biotechnol Prog, 38, e3286.

Appendix A: Supplier Information

Table A1. List of resins and suppliers.

Supplier	Bio-Rad Laboratories, Inc.	Cytiva	JNC Corporation	MilliporeSigma	Sartorius AG	Thermo Fisher Scientific Inc.	Tosoh Biosciences LLC
Resin	Macro-Prep DEAE	Amino Sepharose 6 Fast Flow	Cellufine DEAE A-500	Fractogel EMD DEAE (M)	DEAE Ceramic HyperD F	POROS 50 PI	TSKgel SuperQ-5PW
	Macro-Prep High Q	DEAE Sepharose Fast Flow		Fractogel EMD TMAE (M)		POROS 50 D	TOYOPEARL GigaCap Q-650M
	Nuvia Q	Capto Q ImpRes		Fractogel EMD TMAE Hicap (M)		POROS 50 HQ	TOYOPEARL SuperQ-650S
	Nuvia HP-Q	Q Sepharose XL		Eshmuno Q			
	UNOsphere Q	Q Sepharose Big Beads					
	Nuvia aPrime 4A	Q Sepharose Fast Flow					
		Q Sepharose High Performance					
		Capto adhere					

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Please visit bio-rad.com/Macro-PrepHighQ for more details on Macro-Prep High Q Resin.

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Italy *00 800 00 24 67 23* ***Japan*** *81 3 6361 7000* ***Korea*** *82 080 007 7373* ***Luxembourg*** *00 800 00 24 67 23* ***Mexico*** *52 555 488 7670*
The Netherlands *00 800 00 24 67 23* ***New Zealand*** *64 9 415 2280* ***Norway*** *00 800 00 24 67 23* ***Poland*** *00 800 00 24 67 23* ***Portugal*** *00 800 00 24 67 23*
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