How to improve lentivirus polishing recovery using Capto™ Core 700 resin

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Introduction

Lentiviruses are enveloped RNA viruses belonging to the *retroviridae* family with a particle size of ~ 80–100 nm (Fig 1). They can carry large payloads up to 9 kb, and they can infect both dividing and non-dividing cells. Lentiviruses are one of the most efficient gene transfer vectors and integrate their RNA into the host cell DNA. Lentiviruses are commonly used for chimeric antigen receptor (CAR) T cell therapy to successfully treat cancer. Purification of lentiviral vectors is very challenging due to the low stability of this enveloped virus, which is sensitive to low pH, high salt, temperature, shear forces, and other factors. In the different bioprocess steps from harvest to final sterile filtration, efforts must be made in order to minimize conditions that negatively affect a good physical and infectious titer recovery. Lentivirus purification alternatives can be considered, and the choice depends on differences in purity demands *in vivo* vs *ex vivo* (Fig 2). In this study, we followed alternative B, using capture with weak anion exchange and optimization of the Capto™ Core 700 resin polishing step to maximize infectious recovery.

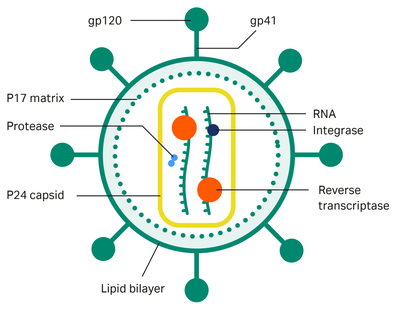

Fig 1. Schematic representation of lentivirus particle structure.

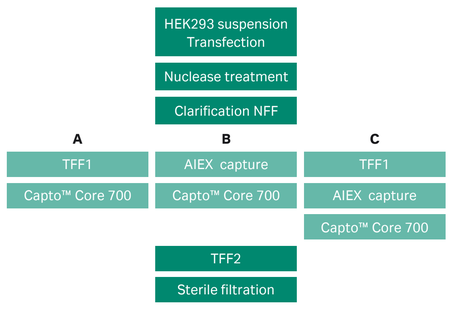

Fig 2. Process alternatives (A, B or C) overview.

Materials and methods

Lentivirus material

In this study, we used lentivirus-GFP material produced in HEK293 cells, treated with DNA nuclease, and clarified by 0.45 µM filtration before anion exchange capture with different anion exchange strengths (Fig 2, process B).
The clarified feeds (~ titers > 1 × 10^9 VP/mL and > 1 × 10^6 TU/mL) were aliquoted and stored at –80°C and applied to anion exchange capture after a slow thawing protocol.

Capture with Capto™ DEAE resin

The capture was performed using a 5 mL HiTrap™ Capto™ DEAE column, which has a resin with a weak anion exchange ligand on dextran surface extenders that was previously reported to result in the highest infectious recoveries (Cytiva). The protocol used for the capture is shown in Table 1. In order to maintain virus infectivity, the lentivirus elution fractions were directly diluted 1:5 into 50 mM Tris-HCl pH 7.0, 7.4, or 8.0, 4% sucrose, and 130 mM NaCl. All runs were performed in duplicates.

Polishing with Capto™ Core 700 resin

The Capto™ DEAE resin eluate was applied to a 1 mL HiTrap™ Capto™ Core 700 column equilibrated in 50 mM Tris-HCl pH 8.0, 7.4, or 7.0. Lentivirus load was approximately 1 × 10^{11} VP in 50 mM Tris-HCl pH 7.0, 7.4, or 8.0, 4% sucrose, and 130 mM NaCl.
The flowrates evaluated were 0.3 mL/min, 0.9 mL/min, 1.4 mL/min, and 1.9 mL/min (3.3, 1.1, 0.7, and 0.5 min residence times). The protocol used for the polishing is shown in Table 2. All runs were performed in duplicates.

Analytics

Physical titer was determined by p24 ELISA (VP/mL) and infectious titer (TU/mL) by a cell-based transduction assay and flow cytometry to measure expressed GFP. All sample analyses were run in duplicate with a reference included in each run. To measure impurities, total protein was analyzed using a Micro BCA assay and total DNA was analyzed by Quant-iT PicoGreen dsDNA Assay (Thermo Fisher Scientific).

Table 1. Protocol for Lentivirus capture with Capto™ DEAE weak anion exchange resin

System: ÄKTA pure™ 25
Column: Capto™ DEAE (HiTrap™ 5 mL)
Flow rate: 5 mL/min

Phase	Composition	Volume
Equilibration	50 mM Tris-HCl pH 8.0, 130 mM NaCl	3–10 CV
Sample loading	Lentivirus feed (load ~ 1 × 10^{11} VP/mL resin)	120 mL
Wash	50 mM Tris-HCl pH 8.0, 130 mM NaCl	10 CV
Elution	50 mM Tris-HCl pH 8.0, 130–650 mM NaCl	25 mL
Wash	50 mM Tris-HCl pH 8.0, 1.3 M NaCl	10 CV
CIP	1 M NaOH	15 CV
Wash	50 mM Tris-HCl pH 8.0, 1.3 M NaCl	15 CV
Re-equilibration	50 mM Tris-HCl pH 8.0, 130 mM NaCl	10 CV

Table 2. Protocol for lentivirus polishing with Capto™ Core 700 resin

System: ÄKTA pure™ 25
Column: Capto™ Core (HiTrap™ 1 mL)
Flow rates: 0.3 mL/min, 0.9 mL/min, 1.4 mL/min, and 1.9 mL/min

Phase	Composition	Volume
Equilibration	50 mM Tris-HCl pH 8.0, 7.4, or 7.0	5 CV
Sample loading	Lentivirus Capto™ DEAE eluate (~ 1 × 10^{11} VP/mL resin) diluted in 50 mM Tris-HCl pH 7.0, 7.4, or 8.0, 4% sucrose, and 130 mM NaCl	8 mL
Wash	50 mM Tris-HCl pH 7.0, 7.4, or 8.0, 4% sucrose, and 130 mM NaCl	7 CV
CIP	1 M NaOH, 30% isopropanol	5 CV
Wash	50 mM Tris-HCl pH 7.0, 7.4, or 8.0	5 CV
Re-equilibration	50 mM Tris-HCl pH 7.0, 7.4, or 8.0	5 CV

CV = Column volume, CIP = Clean in place.

Results and discussion

The capture with the weak anion exchanger Capto™ DEAE resin resulted in approximately 70% physical particle and infectious virus recovery. DNA co-eluted with the lentivirus with only about 20% removal, but most of the HCP came in the flowthrough. The levels of HCP in the eluate fractions were below the limit of detection (Fig 3). To remove the DNA, a polishing step using Capto™ Core 700 resin was evaluated. This resin is used in flowthrough mode and has an unfunctionalized outer shell with a pore size (approx. 700 kDa cut-off) that allows impurities to enter and bind to octylamine ligands inside the bead. Meanwhile, large particles such as lentiviruses flow through the resin. It is important that the DNA is degraded by nuclease treatment in order to enter Capto™ Core 700 beads and be removed.

We wanted to study if altering the pH could improve the infectious recovery for Capto™ Core 700 resin polishing. An overview of the study can be seen in Figure 4. The Capto™ DEAE resin eluates were therefore diluted in buffers with three different pH values before applied to Capto™ Core 700 resin that was equilibrated at the corresponding pH. The recovery of physical particles (p24 ELISA) were similar, but the infectious recovery was significantly improved using pH 7.0 compared to pH 7.4 and pH 8.0 (Fig 5A). Our hypothesis is that the virus envelope integrity, which is critical to infectivity, is better maintained at pH 7.0 in contrast to higher pH values. Higher pH values could shift dissociation equilibriums toward destabilizing the envelope and potential integral biomolecules could be scavenged by Capto™ Core 700 resin, thereby reducing virus infectivity. This lower pH 7.0 was used to investigate the effect of increased flow rate on the infectious recovery. The infectious recovery was similar using up to 1.4 mL/min (0.7 min RT), but at 1.9 mL/min, a small reduction in recovery could be seen (Fig 5B). Impurities levels were below the limit of detection for all pH values and flow rates (data not shown).

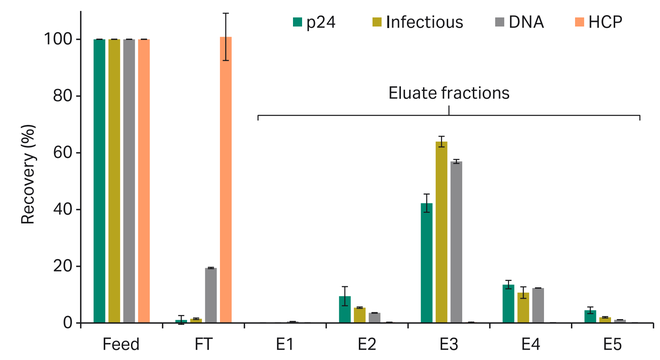

Fig 3. Lentivirus capture with Capto™ DEAE resin using the protocol in Table 1. Clarified feed was applied, and flowthrough (FT) and eluate fractions (5 fractions, E1–E5 detailed in this figure and Table 1) were collected and diluted 1:5 in sucrose containing buffer pH 8.0 (see Figure 4). Virus titer (p24 ELISA and transduction assay) and impurities were analyzed in the different fractions. Recoveries were calculated and plotted in the diagram.

Elution fractions:
E1: 130–390 mM NaCl (10–30% B buffer)
E2: 390–650 mM NaCl (30–50% B buffer)
E3, E4, and E5: 650 mM NaCl (50% B buffer)

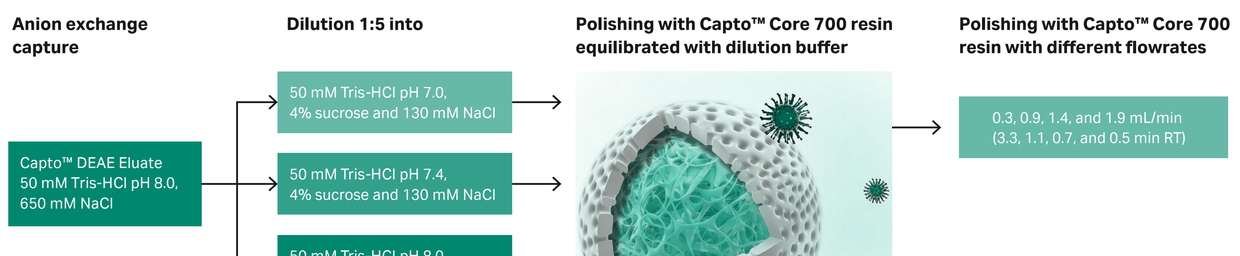

Fig 4. Study overview for evaluation of different buffer pH values and flow rates (pH 7.0 only) for the Capto™ Core 700 resin polishing step.

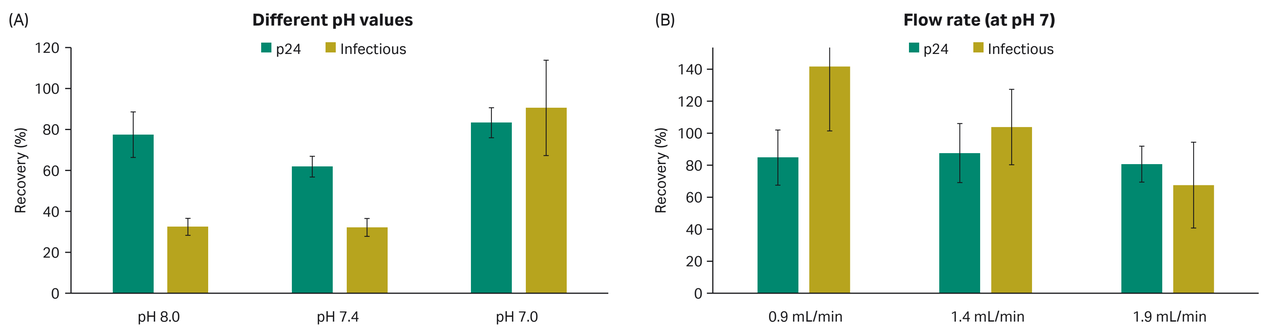

Fig 5. (A) Polishing with Capto™ Core 700 resin at different pH values. Capto™ DEAE resin eluate in 50 mM Tris-HCl, 130 mM NaCl, and pH 7.0, 7.4, or 8.0 was applied on Capto™ Core 700 resin with flow rate 0.3 mL/min (residence time 3.3 min). (B) Polishing with Capto™ Core 700 resin at pH 7.0 and different flow rates. Capto™ DEAE resin eluate in 50 mM Tris-HCl, 130 mM NaCl, and pH 7.0 was applied on Capto™ Core 700 resin using flow rate 0.9 mL/min, 1.4 mL/min, and 1.9 mL/min (residence time 1.1 min, 0.7 min, or 0.5 min). Flowthrough fractions were collected, and physical, infectious virus titer and impurities were analyzed.

Conclusions

- Weak anion exchange (Capto™ DEAE resin) capture resulted in about 70% infectious recovery.
- Capto™ Core 700 resin polishing at pH 7.0 significantly improved infectious recovery.
- Capto™ Core 700 resin polishing can be run as fast as 0.7 min residence time (1.4 mL/min) with retained recovery and impurity removal.
- HCP and DNA (nuclease treatment critical) is below limit of detection after Capto™ Core 700 resin polishing.
- Capto™ Core 700 resin polishing is scalable and enables high load capacity (~ 30 CV) compared to size exclusion (0.1 to 0.3 CV).

Acknowledgement

We acknowledge Adam McLeod and the team at Cytiva/CCRM in Toronto for discussions and for kindly providing the lentivirus feed.

CY36113-03Apr23-PO

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